

COMPARISON OF TECHNOLOGIES TO CONTROL THE
PHYSIOLOGICAL, BIOCHEMICAL AND NUTRITIONAL CHANGES OF FRESH CUT
FRUIT

by

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Abstract

Fresh cut fruits are products with minimal processing, modified by cutting, washing, packaging and refrigeration. The objective of this review is to present an overview of the available technologies for processing fresh cut fruits and technologies that could have some potential to be used in the future due to the increased consumption of fresh cut fruits. Fresh cut products are ready to eat, have high moisture content, have lack of lethal step to eliminate microbial pathogens and have the potential for temperature abuse; all these factors make quality and safety a priority. Fresh cut products sales have increased as a consequence of increased consumption of fruits and consumer attitudes toward convenience.

Fresh cut fruit differ from fresh fruits in that the tissue and cell integrity have may been disrupted resulting in consequences such as changes in ethylene production, respiration, membrane degradation, metabolite accumulation, water loss and microba spoilage. However, the response to cutting depends on other variables such as cultivars, maturity and raw material quality, shape of cut, sharpness of blade, temperature and atmospheric composition. Consequently, there are enzymatic changes that impact the shelf life and quality of these products.

In addition to quality and safety the nutritional value is an important factor too because consumers demand products as close to fresh as possible. The evaluation of the nutrient losses and how to reduce them through different treatments such as the introduction of vitamins and minerals in coating treatments or other treatment has become a priority too.

There is a real need to find alternatives for preservation of fresh-cut fruit in order to minimize the changes that occur in the fruit tissues as a consequence of wounding. Alternatives, combined or modified methods have been proposed such as the use of alternative antibrowning, ethylene scrubbing, heat treatments, alternative antimicrobials, alternative packaging films, vacuum impregnation, osmotic dehydration, high hydrostatic pressure, use of edible coatings, radiation, bio preservation and other technologies.

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CHAPTER 1 - Background Information

Fresh cut Definition

Fresh-cut fruits and vegetables are products that are partially prepared so that no additional preparation is necessary for their use. They are prepared for restaurants, fast food outlets and retail markets. The package can be containers over-wrapped with film, film packages or glass jars (Watada and Ling, 1999). It is defined also as any fruit or vegetable or combination that has been trimmed, peeled, washed and cut into 100% useable product that is then bagged or prepackaged and remains in fresh state (IFPA, 2004).



Figure 1-1 Fresh cut fruit picture

Fresh cut trends

Fresh cut fruits have benefited from the demand for convenience. Retail sales of fresh cut items have grown from under \$100 million in 1990 to \$15 billion in 2006 with a high contribution from the foodservice operators. Although the category of fresh cut fruit initially had many losses due to spoilage and some supermarkets were not sure about giving fresh cut products additional shelf space; the category has moved forward to be the fastest growing category in fresh fruit and vegetables. There has been an increased offering in salads and cut-fruit in the menus of quick service restaurants, school cafeterias, convenience stores and even vending machines (IFPA, 2004). In the first quarter of 2006, fresh-cut fruit represented \$242 million of the total fresh-cut sales, with fresh-cut vegetables representing \$1 billion. The trends for fresh cut sales in billion of dollars are shown in Figure 1-2 (IFPA, 2006).

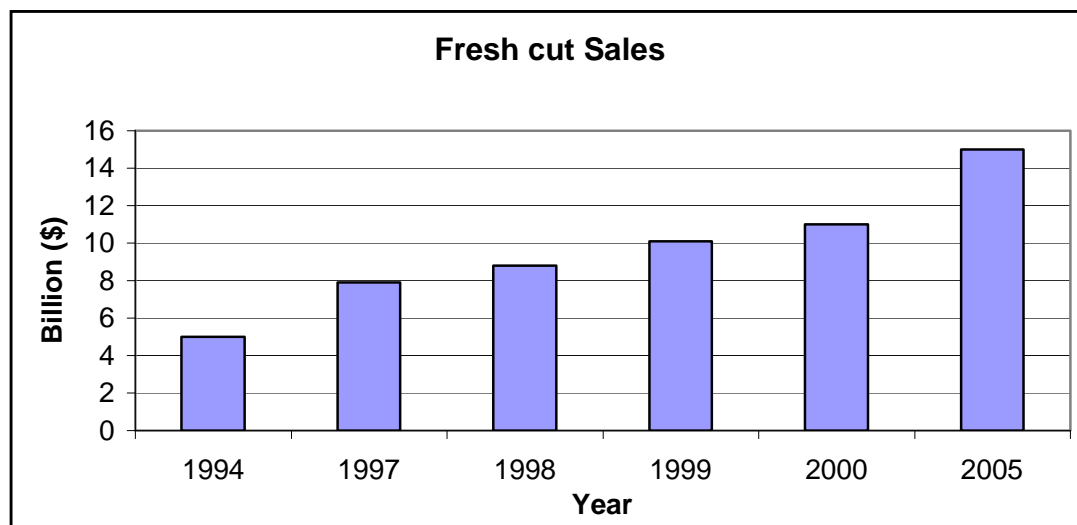


Figure 1-2 Fresh cut sales trend by year in US Dollars, (IFPA, 2006)

Fresh-cut product has been sold at retail since 1940, but was not totally successful because the quality was not predictable and the shelf life was limited. One of the reasons for this was that processors were using blemished product or second quality commodities for fresh cut and the refrigeration chain through distribution was poor. But the improvement in these areas and in new packaging technologies and techniques, the shelf life of products has improved and so has the demand. Fresh cut fruit products are complicated by the nature of fruits in which

softening and other ripening processes continue after harvest (Kader, 2002 and Lamikanra, 2002).

The prospect for fresh cut fruits is supported by trends of obesity and overweight and the desire of people to correct this, the food pyramid recommendations by USDA, which recommends 5 daily servings of fruits and the desire for convenience and healthier snacks.

In the early days fresh cut produce consisted of cellophane wrappers over cardboard trays for products like salads. In the early 1980 polyethylene film was the only breathable film on the market and fresh cut processors started using bags that were designed for other foods such as turkey and meats. But in the late 1980 film companies started looking for new polymers and in the 1990's automatic machines and new films allowed processor to launch branded bags for fresh cut (Lamikanra, 2002). In recent years the use of refrigerated glass jars has been used for fresh cut (Sunkist, 2007). Figure 1-3 shows the types of fresh cut fruit according to the package and sales share.

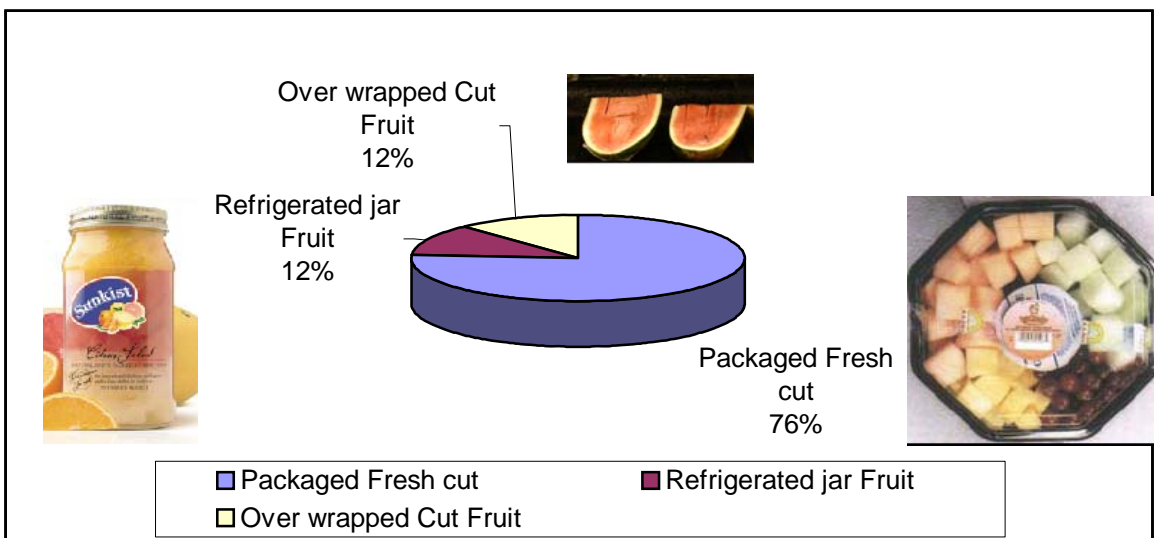


Figure 1-3 Fresh cut fruit sales share by type (IFPA, 2006)

Fresh Cut Unit Operations

Kader (2002) lists as the basic requirements for preparation of fresh cut fruits or vegetables: high quality raw material, strict hygiene and good manufacturing practices, low temperatures during processing, careful cleaning and/or washing before and after peeling, use of mild processing aids in wash water for disinfection or prevention of browning and texture loss, minimize damage during peeling, cutting, slicing and shredding operations, gentle draining to remove excess moisture, correct packaging materials and methods, and correct temperature during distribution and handling.

A basic flow chart of fresh cut products is described in Figure 1-4. The packaging could be done in modified atmosphere or other and cold storage (2-4 °C) under their whole shelf life of 7-10 days. (Barta et al., 2006) Examples of the basic unit operations of a fresh cut pineapple facility are shown in Figure 1-5 and 1-6.

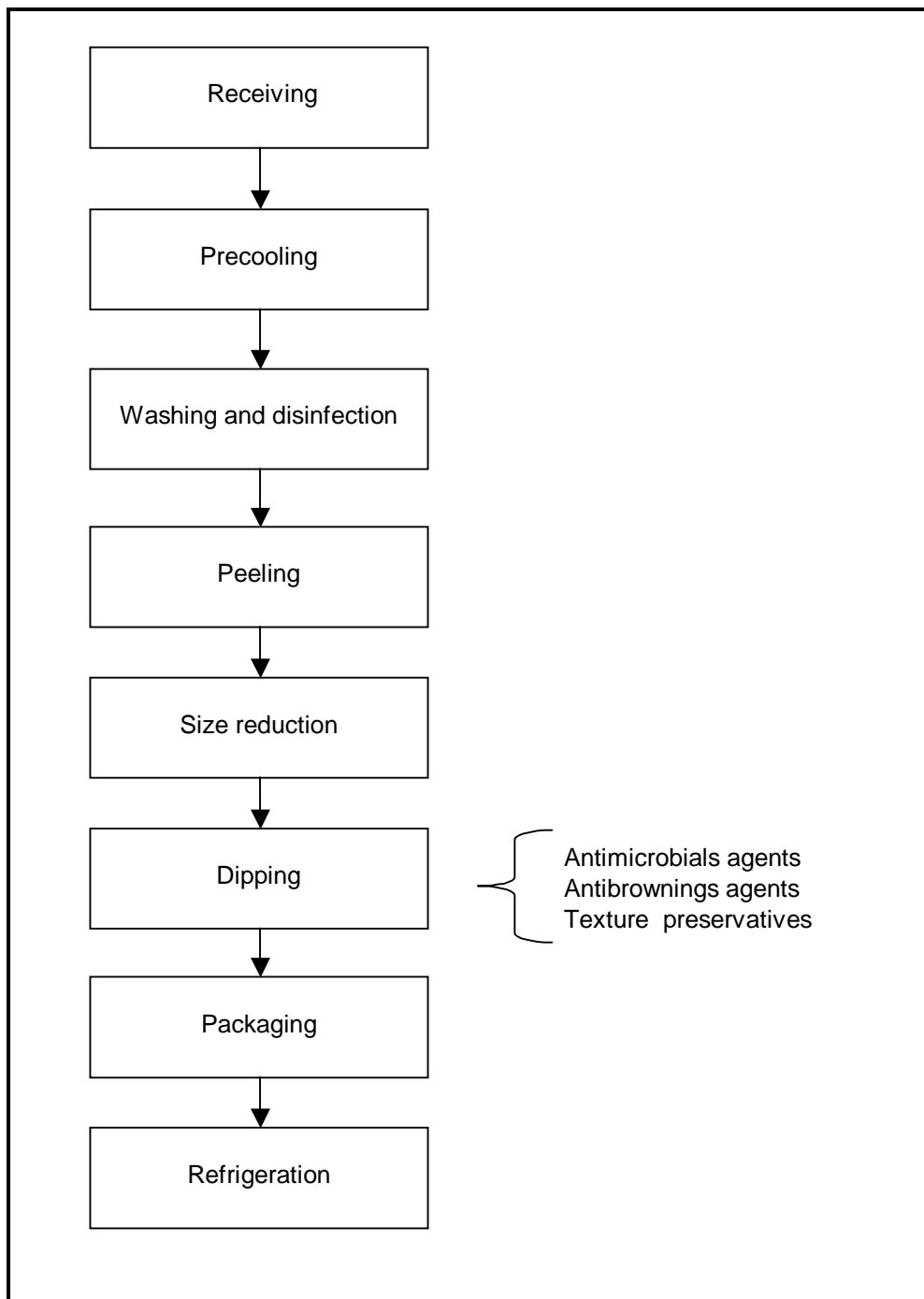


Figure 1-4 Basic process flow chart for fresh cut fruit (Barta et al., 2006)



Harvesting



Fruit wash



Ginaca (removes core and skins)



Trimming and cutting



Figure 1-5 Fresh cut unit operations for pineapple (a)



Scaling / packaging



Bag rinse
Metal detector



Cooling



Packaging
in boxes

Storage and shipping

Figure 1-6 Fresh cut unit operations for pineapple (b)

CHAPTER 2 - Physiological consequences of wounding by cutting

Ethylene Production

Ethylene (C_2H_4) is produced during respiration of fruits and vegetables and acts as a hormone to regulate ripening. Ethylene production is affected by several factors such as fruit maturity, mechanical injuries (wounds, bruises), water stress, temperature and low oxygen (<8%) and >2% CO_2 (Camile, 2000).

Sliced fruit is expected to behave differently from the whole fruit during storage because of the response to wounding of the tissues; but not all fruits respond in the same manner.

In a study done with strawberry and pears (Rosen and Kader, 1989), whole fruits were compared to sliced fruits. The major consequences of slicing strawberries were an increase in CO_2 and C_2H_4 and a loss in firmness but effects were minimized using an atmosphere of 12% CO_2 and $CaCl_2$ dips (1%). Slicing pears caused an increase in CO_2 but not in C_2H_4 relative to whole fruit, loss of firmness was observed as well as browning.

Agar et al. (1999) determined that peeling and slicing kiwifruit caused an increased in CO_2 and C_2H_4 production rates within 2 to 6 hours at 20 °C. And the C_2H_4 and CO_2 production rates of peel were about 2 to 4 times higher than those of unpeeled slices. Peeled fruit and slices had double the C_2H_4 and CO_2 production of whole fruit, which was unchanged during 6 hours at 20 °C or 3 days at 2 °C. Respiration and C_2H_4 production rates increased with temperature.

Respiration

Fruits are living organisms and require oxygen for respiration and carbon dioxide, ethylene and water are bio products. But, in the absence of oxygen, anaerobic respiration or fermentation occurs. Alcohols and aldehyds are the major byproducts of this process with resulting off-flavors (Camile, 2000).

The increase in respiration in wounded plant tissues is thought to be a consequence of elevated ethylene that stimulates respiration. Starch breakdown is enhanced stimulating the tricarboxylic acid cycle and electron transport chain (Brecht, 1995).

In the study by Rosen and Kader (1989) with sliced strawberries and pears, the respiration rate of strawberry slices was greater than that of whole fruit throughout the 8 days storage period. Sliced pears respired at a higher rate than whole pears throughout all the days of storage period. But the differences were only significant during the 7 days at 2.5 °C.

Gorny et al. (2000) compared the respiration and ethylene production of different cultivars of whole and sliced pears (4 cultivars) held at 10 °C and 90-95% relative humidity as shown in Figure 2-1. The difference between whole and sliced pears was evident for Bosc, Anjou and Red Anjou pear slices that had 3%, 65% and 232% greater respiration rates than whole fruits.

Aguayo et al. (2004) studied whole and fresh cut melons and found that wounding by cutting caused an increase in carbon dioxide and ethylene production. The increase was more pronounced at 5 °C than at 0 °C.

Kader (2002) showed a comparison of respiration and ethylene production of fresh cut fruit pieces (Table 2-1), although as mentioned above, there is a strong response by cultivar.

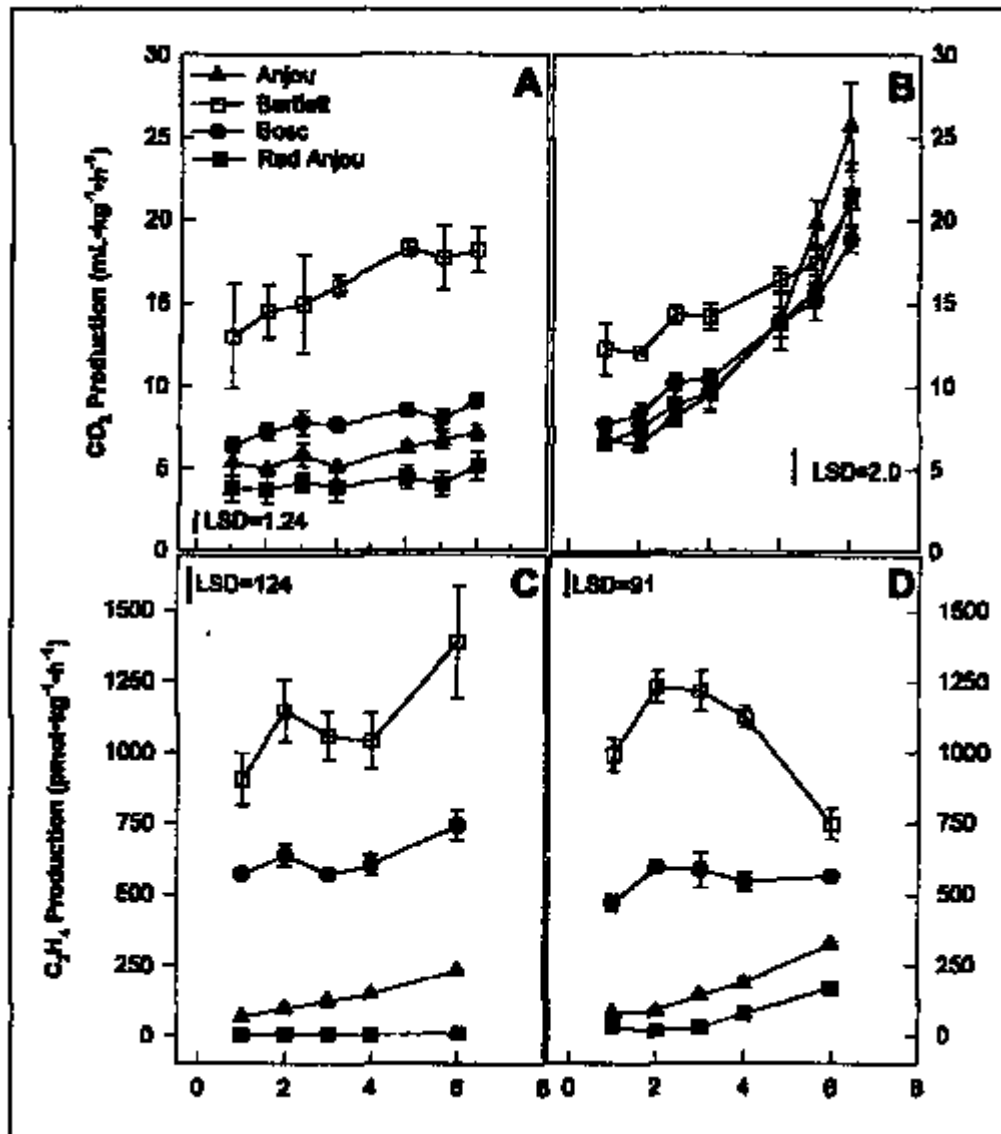


Figure 2-1 Cultivar differences in the respiration and Ethylene production rates of whole pears (A and C) and pear slices (B and D), held at 10° and 90% -95% RH (Gorny et al. 2000)

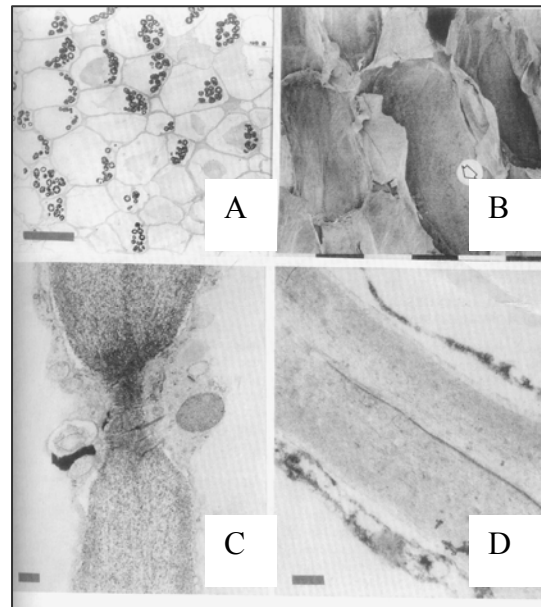
Table 2-1 Ethylene and respiration of fresh cut pieces compared to intact fruit (Kader, 2000)

| Fruit | Stage of Ripeness | Temperature (°C) | Piece size | Respiration compared to intact fruit | Ethylene production compared to intact fruit |
|--------------|--------------------------|--------------------------|-------------------|---|---|
| Apple | Ripe | 2 | Wedge | Increase | --- |
| Banana | Unripe | 20 | 0.4cm | --- | Increase 4x |
| | Ripening | | 0.4cm | Increase | Increase |
| | Ripe | | 4cm. | Same | Same |
| Cantaloupe | Ripe | 20 | 0.2mm | --- | Increase 10x |
| | Ripening | 2 | 2x1 cm cylinder | Same | Same |
| | Ripe | 2 | 2x1 cm cylinder | Same | Same |
| | Ripening | 10 | 2x1 cm cylinder | Same | Same |
| | Ripening | 20 | 2x1 cm cylinder | Increase 2x | Same |
| Kiwifruit | Ripe | 20 | 1cm | Increase | Increase 8x |
| Pear | Ripening | 2 | 1-cm wedge | Same | Same |
| | | 20 | 1-cm wedge | Increase | Reduced |
| Strawberry | Ripe | 2 | Quarters | Same | Same, none |
| | | 20 | Quarters | Increase | Increase 4x |

Membrane degradation

Plant tissue structure can be studied using microscopy (optical, electron, and atomic microscopy) and other imaging techniques with other localization techniques (x-ray, microanalysis, etc). The visualization of the true tissue structure is important because can help to explain the degradative process, mainly on the textural behavior of the fruit tissues (Alzamora et al., 2000 and Soliva-Fortuny et al., 2003).

Alzamora et al. (2000) showed some microscopic observations of some fresh fruits: mango, strawberry, papaya and mango (Figure 2-2) to illustrate that plant tissues are composed of different types of cells exhibiting different turgidity, osmotic pressure, elasticity, size and composition and some physical and chemical properties depend on maturity of the fruit.



Cell wall detail for fruits: A-mango, B-strawberry, C-papaya, D-mango

Figure 2-2 Examples of cell tissues for fresh fruit (Alzamora et al., 2000)

Membrane deterioration in fresh cut produce results in decompartmentation of cellular structure and organization and loss of normal cellular function. These changes cause secondary effects of the membrane deterioration such as tissue browning, production of off odors, production of oxygen free radicals, enzymatic degradation of membrane components and production of free fatty acids by the enzymes lipid acyl hidrolase and phosholipase D from membrane lipids (Lamikanra, 2002).

Soliva-Fortuny et al. (2002; 2003) studied the effect of minimal processing and modified atmosphere packaging on the textural and structural properties of fresh cut pears and apples using texture and microstructure observations (Figure 2-3). They believe that CO₂ can be toxic in high concentrations and initiate physiological disorders, because enhances acidity when

dissolved in the cell medium, being one of the main causes of undesirable sensory changes and as consequences the tissue structure is seriously damaged. The micrograph of the tissues of fresh apples compared to fresh cut apples. In (A) fresh apples, in (B) fresh cut apples packed in LOP (low oxygen permeability) bags under 100% N₂ atmosphere stored 45 days at 4 °C showing some intracellular spaces and (C) fresh cut pear packed in modified atmosphere bags under 2.5% O₂ and 7% CO₂ atmosphere and stored for 45 days at 4 °C. Image C in Figure 2-3 shows an inundation of extra cellular spaces that were correlated to the texture data that was collected indicating softening of the tissue.

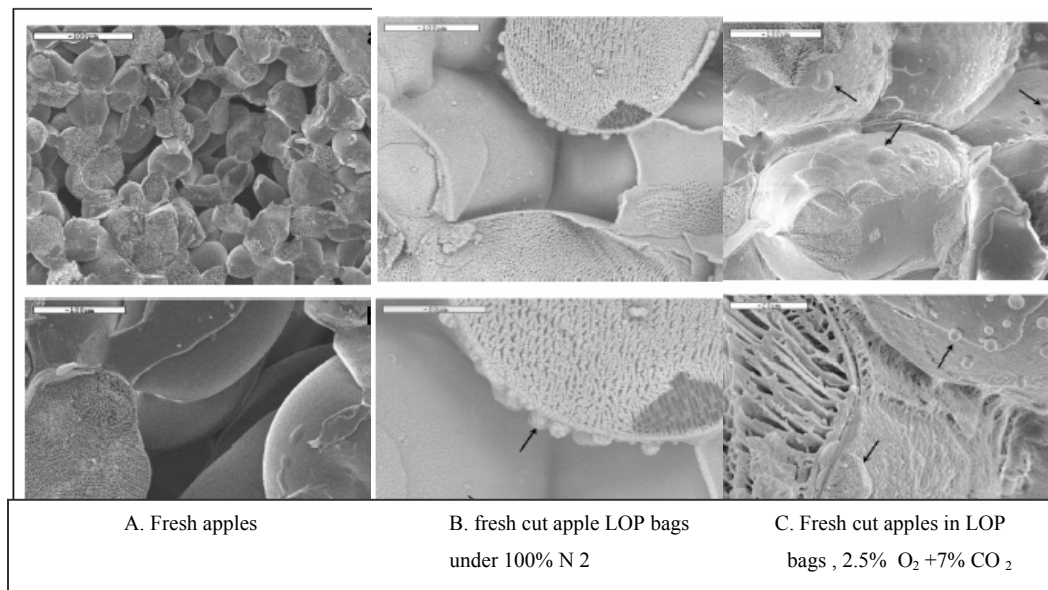


Figure 2-3 Cell tissues micrographs of fresh cut apples under three different atmospheric conditions (Soliva-Fortuny et al., 2003)

Oxidative browning

As a result of cutting and the disruption of compartmentation of the cells, discoloration occurs at the cut surface and when the cells are broken, substrates and oxidases come in contact. Oxidative browning at the cut surface is the limiting factor in storage of many fresh cut fruits (Brecht,1995). Oxidative browning is usually caused by the enzyme polyphenol oxidase (PPO),

which, in the presence of O₂, converts phenolic compounds in fruits and vegetables into dark colored pigments (Beaulieu and Gorny, not dated).

The reaction of enzymatic browning can be catalyzed by the enzyme polyphenol oxidase in two steps: 1. Hydroxylation of monophenols to o-diphenols and 2. Oxidation of o-diphenols to o-quinones. The reactions are shown in Figure 2-4.

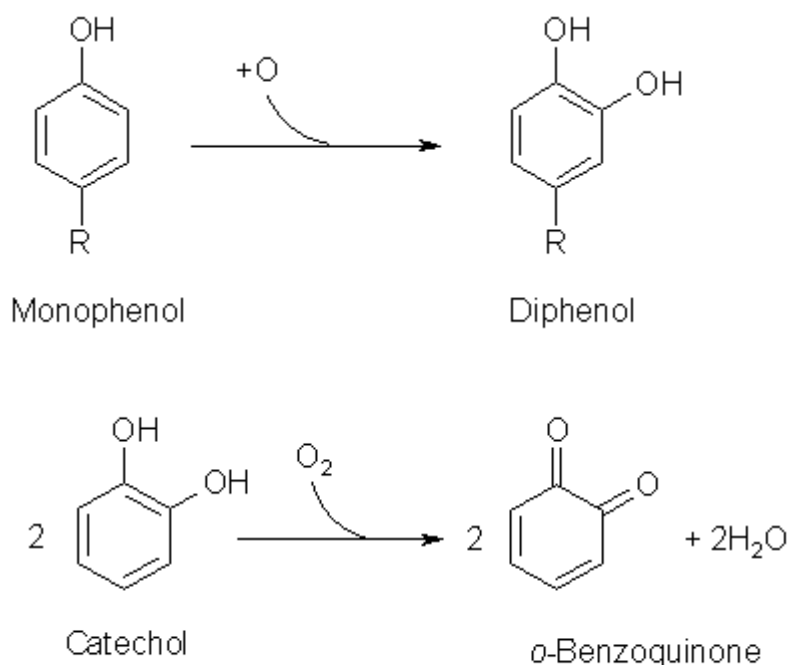


Figure 2-4 Enzymatic browning reactions catalyzed by polyphenoloxidase (Marshall et al., 2000)

Cultivars differ in the degree of browning for example in a study with twelve cultivars of apple and found that slices of Cortland, Empire, Golden Delicious, New York 674 and Delicious showed the least browning after 3 days at 2 °C exemplifying how important it is to select the proper cultivar to control browning (Watada and Qi, 1999).

Enzymatic browning requires oxygen, enzyme, copper and a substrate. There are different strategies to control browning such as reduced concentration of oxygen, acidification and the use of reducing agents. This information is discussed in Chapter 6.

Secondary metabolites synthesis

Since the fresh cut process involves wounding the tissue, the tissue appears to respond with a defense and healing reaction by synthesis of secondary compounds. The secondary compounds depend on the plant species and tissue involved, but these compounds might affect aroma, flavor, appearance, nutritive value or safety of fresh cut products (Brecht, 1995).

Phenolic accumulation is one of the most studied phenomena in response to wounding. This causes the oxidation of endogenous phenolics as a consequence of cell membrane breakdown, allowing the phenolic to mix with oxidative enzymes systems, which are normally separated by membranes. Also, the cells close or adjacent to the injury produce more phenolics as they attempt to repair the damage. The phenolic accumulation is initiated by the increased activity in phenylalanine ammonia lyase (Lamikanra, 2002).

Water loss

Plant tissues are in equilibrium with an atmosphere at the same temperature and RH. But when the fruits are cut or peeled the tissues are exposed and this increases the water evaporation rate. The rate of water loss between intact and wounded plant surfaces varies according to the commodity (Watada and Qi, 1999).

Aguayo et al. (2004) found in a study of the metabolic behavior and quality changes of whole and fresh processed melon that weight loss was variable depending on the cut. The weight loss was 1.1% in slices, 1% in cylinders and 0.6% in trapezoidal sections. These results are related to the greater exposed area of slices and the smaller piece size for cylinders. For the 2 varieties (Piel de Sapo and Amarillo) the weight losses were lower at 0 °C (0.85%) than at 5 °C (1.28%).

Agar et al. (1999) working with kiwifruits found that mass loss was highest in peeled slices and lowest in intact whole fruit stored for 3 days at 20 °C. Fresh cut slices had more water loss since they do not have the protective epidermal cells and surface area/mass rate was increased. These results are shown in Figure 2-5.

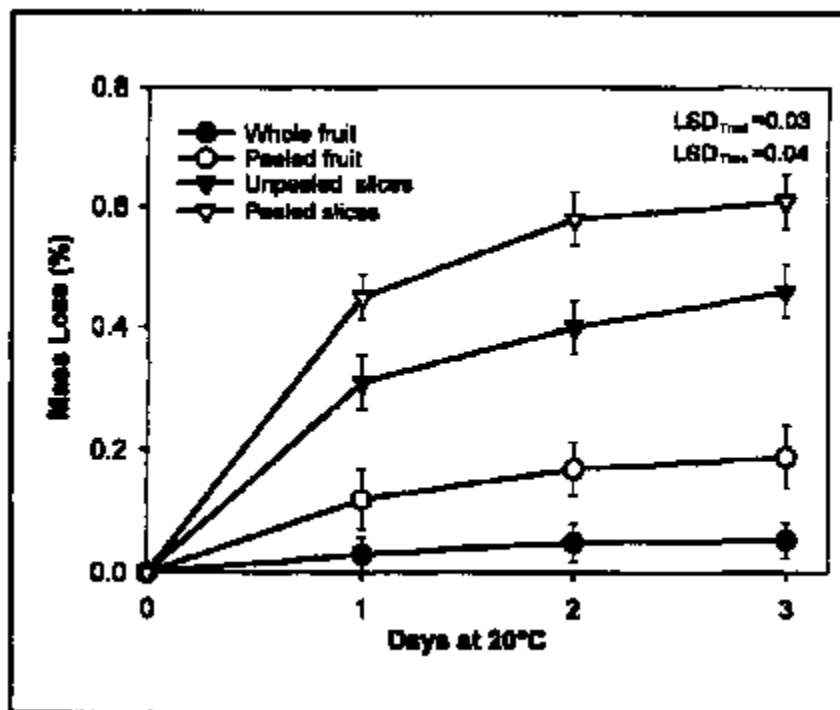


Figure 2-5 Mass loss (%) of Kiwifruits whole, peeled, unpeeled slices, and peeled slices held at 20 °C and 60% RH (Taken from Agar et al., 1999)

Water loss as “juice leakage” from pulp pieces is another important factor of deterioration in fruit such as pineapple. Marreno and Kader (2005), found the amount of leakage depended on the variety of pineapple. After 15 days of storage the Premium Select pineapple pieces leaked about one third the volume of the variety SC 3620, which leaked up to 40mL/kg. This juice leakage was not reduced neither with refrigeration nor atmosphere modification.

Susceptibility to Microbial spoilage

Microorganisms such as mesophilic bacteria, lactic acid bacteria, coliforms, yeasts and molds have been found to be actively growing in packaged fresh cut fruits and vegetables. Increases in microbial populations are related to increased respiration rates with time in storage

and the factor that damaged tissue and broken cells provide nutrients and a protected environment for growth of most types of microflora (Lamikanra, 2002).

The microbiological risks for fresh cut fruit are several: 1. Refrigeration is used to maintain quality but is not a killing step, 2. While modified atmosphere might inhibit the growth rate of many organisms some other pathogens may actually thrive under these conditions, 3. Operations such as trimming and washing may not only eliminate the presence of normal indigenous spoilage organisms but might introduce pathogens or give them a competitive advantage for growth and 4. Fresh cut fruits are consumed raw (Hurst, 1995).

The natural fruit barriers such as the peel, rind and skin prevent microorganisms from entering fruits. However, breaks in these barriers caused by punctures or damage during handlings or the cutting can allow pathogens such as *Escherichia coli* 0157:H7 to enter and potentially grow. Fatemi et al (2006) studied the ability of *Escherichia coli* 0157:H7 to penetrate and grown within punctures of fresh cut Golden Delicious apples. The fresh cut surfaces permitted up to 2.8 mm penetration and the population increased 3 logs after 48 hours. These findings show the importance to control the temperature of the fruit and rapid speed of processing as well as the use of sanitizing treatments.

The guide to minimize food safety hazards for fresh fruit and vegetables (FDA, 2007) includes as major areas of concern: 1) water quality, 2) manure/municipal biosolids, 3) worker hygiene, 4) field, facility, and transport sanitation, 5) Production controls and sanitation and 6) traceback program. Growers, packers, shippers and processors should consider the variety of physical characteristics of produce, but the practices that affect the potential sources of microbial contamination associated with their operation, and decide on which combination of good agricultural and management practices to follow.

The appropriate handling during harvesting and post harvesting can reduce the risk of contamination during processing. If fruit were contaminated with pathogenic microorganisms during these steps even disinfectant wash cannot totally assure the safety. This is why the cleaning operations of field bins, storage chambers and any other source of cross contamination are so important. Then, at the processing plant adherence to Good Manufacturing Practices (GMP's) and implementation of Hazard Analysis of Critical Control Points (HACCP) is advised (Barta et al., 2006).

Several food borne outbreaks (*Salmonella*, *Campylobacter* and *E. Coli* 0157:H7) have been associated with the consumption of cantaloupe and other melons. Mature cantaloupes have a pH of 6-7 and are an excellent substrate for growth of bacteria especially at warmer temperatures. Cut melon is considered as a hazardous food that is capable of supporting the growth of pathogenic microorganisms. The recommendation is to obtain cut melons from regulated food sources and to ensure keeping temperature at 45 °F or below (Luna-Guzman, 1997).

Low temperatures are necessary to reduce respiration rates, retard deterioration but also to retard microbial growth. Ukuku and Sapers (2007) determined the effects of a waiting period at 22 °C before refrigerating fresh-cut watermelon, cantaloupe and honeydew pieces inoculated with *Salmonella* to verify the survival. Whole cantaloupes, honeydew melons and watermelons were washed with water, and fresh-cut pieces from individual melons were prepared and inoculated with a five strain mix of *Salmonella* at 10^5 cfu/ml. Populations of *Salmonella*, aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* ssp. in fresh-cut melons left at room temperature for up to 5 hours before refrigeration were significantly higher than populations in fresh-cut melons stored at 5 °C immediately after preparation. The study showed that holding freshly prepared, contaminated fresh-cut melon pieces at 22 °C for 3 hours or more prior to refrigerated storage would increase the chance of *Salmonella* proliferation, especially if the fresh-cut melons were subjected to temperature abuse.

Lamikanra et al. (2005) point out the importance of the role of *Pseudomonas* in postharvest rot of fresh produce. *Pseudomonas* bacteria are normally on the surface of produce and have the ability to cause spoilage due to their ability to produce depolymerases including pectinases, proteinases, cellulases and lipases.

The survival and growth of pathogens on fresh produce are influenced by several factors; these factors are the organism, produce item, and environmental conditions in the field including storage conditions. In general, pathogens will survive but not grow on the uninjured outer surface of fresh fruits or vegetables, mainly because of the natural barriers of the plant. One exception is the reported growth of *E. coli* O157:H7 on the surface of watermelon and cantaloupe rinds as shown in Table 2-2. Survival of food borne pathogens on produce is significantly enhanced once the protective epidermal barrier has been broken either by physical damage or by degradation by plant pathogens (bacteria or fungi). These conditions may also promote the multiplication of

pathogens, especially at nonrefrigerated temperatures. Microorganisms often survive at refrigerated temperatures even though these conditions reduce their ability to multiply but there still are some exceptions such as the psychrotrophic pathogens including non-proteolytic *C. botulinum*, *L. monocytogenes* and *Y. enterocolitic*. A high population of nonpathogenic bacteria is potentially another barrier to reduce the risk of food borne illness from fresh-cut products. These bacteria do not necessarily prevent the growth of pathogens but they do provide indicators of temperature abuse and age of the produce by causing detectable spoilage (FDA, 2001 (a)). Table 2-2 and 2-3 present the incidence, growth, and survival of pathogens in Fresh and Fresh-Cut Produce in melons and for other fruits.

Table 2-2 Survival and growth of pathogenic bacteria on raw melons (FDA, 2001(b))

| Pathogen | Fruit | PH | Mode of inoculation | Storage conditions | Temperature °C | Initial counts (log ₁₀ CFU) | Incubation Time | Final counts (log ₁₀ CFU) | Units |
|---|-------------------------|------|--|---|----------------|--|----------------------|--------------------------------------|---------------------|
| <i>Campylobacter jejuni</i> | Watermelon cubes | 3 | Spot inoculation, cells suspended in saline. 0.02 ml inoculated per cube. | 24 cm ² cubes with 0.05 ml of lemon juice added per cube. Stored in covered sterile stainless-steel trays. | 25-29 | 2.9 | 6 hours | 2 | CFU/cubes |
| <i>Campylobacter jejuni</i> | Watermelon cubes | 5.5 | Spot inoculation, cells suspended in saline. 0.02 ml inoculated per cube. | 24 cm ² cubes without lemon juice. Stored in covered sterile stainless-steel trays. | 25-29 | 2.7 | 6 hours | 2.1 | CFU/cubes |
| <i>Escherichia coli</i> O157:H7 (4 strains) | Watermelon rind surface | - | Spot inoculation cells diluted in 0.1% peptone | Melons were held in covered plastic boxes with 93% RH. | 25 5 | 5.2 5.2 | 21 days 8 days | ~7.1 <1.0 | CFU/cm ² |
| <i>Escherichia coli</i> O157:H7 (4 strains) | Watermelon cubes | 7.01 | Cells suspension diluted in 0.1 % peptone | Cubes placed in sealed stomacher bags and incubated | 25 5 | 3.0 ~3.1 | 34 hours 34 hours | ~7.0 ~3.1 | CFU/cm ² |
| <i>Salmonella</i> (5 serotypes) | Cantaloupe, cubes | 6.67 | Cell suspension diluted in Butterfield's phosphate butter | Cubes placed in open stomacher bags and incubated aerobically | 23 5 | 2.0 2.0 | 24 hours 24 hours | ~7.2 ~1.6 | CFU/g |
| <i>Shigella Flexneri</i> | Watermelon cubes | - | Spot inoculation, cells suspended in saline. 1 drop inoculated per cube | 12 cm ² cubes inoculated and stored in sterile covered glass trays | 22-26 | 2.8 | 6 hours | 4.5 | CFU/cm ² |
| <i>S. Sonnei</i> | Watermelon, whole | - | 30 ml of inoculum was injected into the whole watermelon through the stem scar | 30ml of inoculum was injected and incubated | 30 22 | 2.0 2.0 | 2 days 4 days | ~9.0 ~9.0 | CFU/g |

**Table 2-3 Survival and growth of pathogenic bacteria on raw fruit other than melons
(FDA, 2001 (b))**

| Pathogen | Fruit | PH | Mode of inoculation | Storage conditions | Temperature °C | Initial counts (log ₁₀ CFU) | Incubation Time | Final counts (log ₁₀ CFU) | Units |
|---|--------------------------|-----------------|--|--|----------------|--|------------------------------|--------------------------------------|-----------|
| <i>Campylobacter jejuni</i> | Papaya, cubes | 3.0 | Spot inoculation, cells suspended in saline 0.02 ml inoculated per cube | 24 cm ² cubes with 0.05 ml of lemon juice added. Stored in sterile stainless steel trays with covers | 25-29 | 3.3 | 6 hours | <1.0 | CFU/ Cube |
| <i>Campylobacter jejuni</i> | Papaya, cubes | 5.0 | Spot inoculation, cells suspended in saline 0.02 ml inoculated per cube | 24 cm ² cubes without 0.05 ml of lemon juice added. Stored in sterile stainless steel trays with covers | 25-29 | 2.8 | 6 hours | 1.7 | CFU/ cube |
| <i>Escherichia coli</i> O157:H7 | (Golden delicious, | 3.8 4 | Cell suspension in tryptone soy broth, inoculated into sample | Ground apples were stored in a plastic stomacher bag | 4 10 25 | ~7.5 | 18 days 12 days 5 days | ~7.2 ~6.8 ~8.2 | CFU/ ml |
| <i>Escherichia coli</i> O157:H7 (2 strains) | Orange (Hamlin, peeled) | 6-6.5 | Spot inoculation, 20 ul inoculated and spread over 1/8 of total surface area. Inoculum suspended in Butterfield's phosphate buffer | Inoculated fruit were individually packed in perforated plastic container | 4 8 24 | 3.5 | 14 days 14 days 1 day | ~3.2 ~2.5 ~7.5 | CFU/ g |
| <i>Listeria monocytogenes</i> (2 strains) | Orange (Hamlin, peeled) | 6.0 - 6.5 | Spot inoculation, 20 ul inoculated and spread over 1/8 of total surface area. Inoculum suspended in Butterfield's phosphate buffer | Inoculated fruit were individually packed in perforated plastic container | 4 8 24 | 3.9 | 14 days 14 days 1 day | ~4.0 ~3.5~ 5.5 | CFU/ g |
| <i>Staphylococcus aureus</i> (2 strains) | Orange, (Hamlin, peeled) | 6.0 - 6.5 | Spot inoculation, 20 ul inoculated and spread over 1/8 of total surface area. Inoculum suspended in Butterfield's phosphate butter | Inoculated fruit were individually packed in perforated plastic container | 4 8 24 | ~2.8 | 14 days 14 days 1 day | ~2.0 ~2.0 ~3.5 | CFU/ g |
| <i>Shigella</i> (3 species) | Papaya, cubes | 5.6 9 | Spot inoculation, cells suspended in saline. 1 drop inoculated per cube | 12 cm ² cubes, inoculated and stored in covered glass trays | 25-27 | 2.0-2.4 | 6 hours | 3.8-4.2 | CFU/ Cube |

CHAPTER 3 - Variables affecting response to cutting of fruits

Species and variety

The cultivars of fruits and vegetables are routinely screened and selected for specific functional properties to be able to yield product of extended shelf life with fresh-like quality. Processors of fresh cut fruits select cultivars that evaluated under current cultural, postharvest and distribution practices meet the need and specifications for the intended product. The criteria to develop or to select cultivars for such products should be: product specifications identification, shelf life need and limitations, identification of other product that can be derived from the same production lot (Romig, 1995).

Several studies in literature compared the shelf life of fresh cut fruit for different cultivars of fruits showing the importance of this variable on the selection of raw material for fresh cut products.

According to the study of Gorny et al (2000) with four cultivars of pears (Bartlet, Bosc, Anjou and Red Anjou), Bartlett pears had longer shelf life than the others. Others variables determined to be important factors in this study were ripeness stage, fruit size and storage time after harvest.

In another study of Gorny et al. (1999) the response of thirteen cultivars of peaches and eight cultivars of nectarines fresh cut slices under controlled atmosphere and chemical treatments varied between 2 and 12 days at 0 °C. Cultivar, storage atmosphere and post cutting dips of ascorbic acid and calcium lactate affected the shelf life of fresh cut peach and nectarine slices. Being the Cultivar and appropriate maturity at harvest, followed by ripening to appropriate firmness and proper storage temperature (0 °C) and relative humidity (90-95%) were the most important factors on the shelf life of the slices of both fruits.

Lamikanra, et al. (2003) studied different cultivars of cantaloupe and found that cultivars with extended postharvest shelf life as whole fruit had lower volatile aroma compounds and the shelf life as whole fruit was not necessarily related on the quality of the cut fruit.

Aguayo et al. (2004) studied four types of melons processed as fresh cut in different shapes and temperatures. They found differences in response of the cultivars studied to temperature and as a consequence increased translucency and softness.

Besides the influence of cultivars, there are other factors that influence the quality of fresh cut products such as soil, irrigation, and growing conditions. Bett-Garber, et al. (2006) studied the influence of soil type and storage conditions on sensory quality of fresh cut cantaloupe. The two types of soil researched were sandy loam versus heavy clay soil. The melons grown in sandy loam were lower in sweet aromatic and sweet taste and higher in moisture release and fermented flavor. An increase in peroxidase activity was observed in fruits produced in sandy loam soil but decreased in fruits produced in clay soil.

Crisosto et al. (1997) studied orchard factors affecting postharvest stone fruit quality. They recommended the evaluation of preharvest factors and their influence on fruit quality. Factors such as nitrogen levels fertilization can influence different responses on the fruit as high nitrogen levels induces poor visual red color development. Variable fruit gas exchange (CO_2 and C_2H_4) and cuticle thickness varied with nitrogen rates. The relationship between fruit nitrogen concentrations and fruit susceptibility to brown rot has been extensively studied on stored nectarines fruits. Another factor observed to have some influence was a high light environment (outside canopy), that resulted in a longer shelf life than fruit (peaches and nectarines) under a low light environment (inside canopy). Summer pruning and leaf pulling around the fruit increases fruit light exposure and if done properly, can increase fruit color without affecting fruit size and soluble solids contents. But excessive leaf pulling done too close to harvest can reduce both fruit size and soluble solids in peaches and nectarines. Another observation is that a more shaded inner canopy caused a greater incidence of internal browning (IB) than fruit from the high light, outer canopy positions. In regards to other factors, some of the research done on the effect of foliar nutrient sprays suggests having little effect on fruit quality. Irrigation deficit timing is believed to have much influence, such as an increase in fruit defects in peaches. The effect of different irrigation regimes on fruit weight and soluble solids concentrations has been studied in peaches. Other factors studied have been girdling and the influence on split pits and crop load and the influence in fruit size.

Raw Material Quality

Although processing permits the use of fruit, which may not be visually acceptable for fresh market, it is generally recognized that raw product quality for fresh cut should be high to insure a good quality fresh cut product.

A study to compare fresh cut melons from good quality areas with melons that showed externally ground spots and melons with sunburn areas determined that common external defects can impact the quality of fresh-cut melon pieces. Using pulp from beneath sunburned areas should be avoided, and pieces from ground spot areas showed softer pieces than undamaged area (Cantwell and Portela, S., 1998).

In order to detect the quality of the fruit used for fresh cut the industry is using technologies to sort the fruit and avoid internal defects. Near-infrared spectroscopy (NIR) has been used since the 1970s for the analysis of composition of low moisture food products. However, only in the last 10-15 years has NIR been successfully applied to the analysis of high moisture products such as fruit. NIR is a form of vibrational spectroscopy that is sensitive to the presence of molecules containing C-H (carbon-hydrogen), O-H (oxygen-hydrogen), and N-H (nitrogen-hydrogen) groups. Therefore, constituents such as sugars and starch (C-H), moisture, alcohols and acids (O-H), and protein (N-H) can be quantified in liquids, solids, and slurries. There has been a lot of research for NIR analysis of tree fruit. NIR has been used for the measurement of fruit juice, flesh, and whole fruit (Ozanich, 1999).

Bruising is a very important cause of rejection of fruit. Xing and Baerdamaker (2005) used visible and NIR to detect fresh bruises by predicting the softening of the apple tissue.

Physiological maturity

Postharvest physiology considers “mature” and “ripe” as different terms. “Maturity” is the stage at which a commodity has reached a sufficient stage of development that after harvesting and postharvest handling (including ripening, where required) its quality will be at least the minimum acceptable. “Ripe” fruit is fruit at the peak for texture and flavor; it is ready to

eat. The maturity index of a commodity indicates if a commodity is mature and has to be a preferable objective (Kader, 1992).

Climacteric fruits are the fruits that show a large increase in CO₂ and C₂H₄ (ethylene) production rates coincident with ripening. But nonclimacteric fruits do not show change in their generally low CO₂ and C₂H₄ production rates during ripening (Kader, 1992). The physiological maturity of fruits and vegetables impacts the wounding response especially for climacteric fruits (Lamikanra, 2002 and Kader, 2002).

There are many methods to determine the maturity of a fruit. Some examples are: elapsed days from full bloom to harvest (apples, pears), development of abscission layer (melons, apples), surface morphology and structure (cuticle formation on grapes, netting on some melons), size (all fruits), specific gravity (cherries, watermelons), shape (banana, mangoes), texture (apples, pears, stone fruits), external color, internal color and structure, compositional factors (starch, sugar, acid, juice, astringency, etc.) (Kader, 2002).

As cantaloupe (a climacteric fruit) matures on the plant, the abscission layer where the stem (peduncle) attaches to the fruit begins to separate and this separation is called slip. This separation is an indicator of full ripeness and harvest time. Beaulieu et al. (2004) studied cantaloupe harvested at 4 distinct maturities stages ($\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and full slip). The cantaloupes were cut into cubes, stored and evaluated by trained sensory panelists and performing texture analysis at different periods of time. The fruity and sweet aromatic flavor was significantly less intense in the $\frac{1}{4}$ slip cubes compared with $\frac{1}{2}$ and $\frac{3}{4}$ slips maturities. Cubes were harder in $\frac{1}{4}$ slip cube with both sensory and instrumental method. Fresh cut cantaloupe cubes with desirable sensorial attributes were the ones prepared with fruit harvested with $\frac{1}{2}$ slip or more but not from $\frac{1}{4}$ slip. The effect of fruit ripeness was investigated by Gorny et al. (1998) in fresh cut peaches and nectarines slices. Ripeness was evaluated by analytical firmness measurement. Peach and nectarine slices from mature-green fruit (>40-53 N fresh firmness) had the longest shelf life (8 days at 0 °C) for peaches and 8 days at 0, 5, or 10 °C for nectarines. Slices from mature green peaches and nectarines and partially ripe peaches (>27-40 N flesh firmness) failed to soften to acceptable eating quality at 0 or 5 °C. Overripe peach and nectarine fruits (0-13 N flesh firmness) were organoleptically acceptable at the time of cutting but shelf life was only 2 days or less for peaches and 3 to 6 days for nectarine at 0, 5 or 10 °C. The optimal ripeness for preparing fresh cut peach slices was the ripe stage (>13-27 N flesh firmness) with a shelf life of 6 days and

for fresh cut nectarine slices was the partially ripe (>27-49 N) or ripe stage (>13-27 N flesh firmness) at 0 °C with a shelf life of 8 days with good eating quality.

In another study with fresh cut conference pears (Soliva-Fortuny et al., 2004), the maturity was also determined with a texture analyzer. The selected maturities corresponded to 63 N (mature green), 44 N (partially ripe), and 31 N (ripe). The state of ripeness that better kept the fresh-like quality during at least 14 days was the pears at partially ripe maturity (44 N) with a combination of dips in solutions (ascorbic acid and calcium chloride) and O₂ atmosphere control in the package.

Beaulieu, and Lea. (2003) studied the volatile and quality changes in store fresh cut mangos prepared from “firm ripe” and “soft ripe “ mangos stored in clamshell containers and passive modified atmosphere packaging (MAP). Firm ripe fruit processed had lower Brix, slightly inferior color quality and initial aroma and high terpene levels indicating that it was not ripe enough to deliver an optimum product to consumers even though storage life was greater than soft ripe. But also soft ripe cubes were processed when slightly too ripe since they showed tissue damage, mushiness even though the brix and aroma were superior.

Whereas most fruit and vegetables are better suited to minimal processing in less mature physiological stages, some products such as bell peppers (refers to the actual fruit of the capsicum plant), may be most suitable at more advanced stages of maturity (Lamikanra, 2002). Saftner et al. (2006) compared orange and green fleshed honeydew melons and concluded that fresh cut chunks from full slips melons had higher analytical and sensory quality characteristics but higher microbial counts and lower shelf life stability compared to the ones from commercially mature fruit.

Severity of Wounding

Shape of cutting:

Fresh cut fruits are cut in a wide variety of shapes and the cutting shapes influence the degree of damage of wound. Some shapes cause more damage to the tissue and several studies had compared the shape as in Rivera-Lopez et al., (2005), cubes and slices of papaya were compared at two different temperatures (10 °C and 5 °C) and was observed that the slices presented a slight advantage over cubes when comparing total soluble solids content, weight

loss, and overall quality index at both temperatures and more benefit was observed at lower temperature, this was related to the area damaged by the cut.

Aguayo et al. (2004) compared whole melon with slices, trapezoid and cylinder cuts. Firmness was affected by the shape as well as water evaporation. Cylinders showed good firmness retention, followed by trapezoidal; water evaporation and softness sharply increased in slices (higher surface area). Cylinders showed higher translucency, whereas slices showed lower translucency. Trapezoidal sections showed a behavior intermediate between cylinder and slices.

Artés-Hernandez et al. (2006) studied the effect of the cut type and temperature on the shelf life of fresh cut lemons. The cut types used were wedges, slices, $\frac{1}{2}$ and $\frac{1}{4}$ slices of Lisbon lemons stored at 0, 2, 5 and 10 °C in glass jars. Based on sensory analysis, the four cut types remained marketable for up to 7 days at all tested temperatures, but only the wedges, slices and $\frac{1}{2}$ slices stored at 0, 2 and 5 °C preserved their sensory attributes for up to 10 days. Ethanol was found to increase up to three fold in slices, $\frac{1}{2}$ and $\frac{1}{4}$ slices after 10 days at 10 °C.

Angle of cut:

Another study compared the angle of cut for sliced banana. It showed that an increase in angle caused an increase in ethylene production and respiration rates and this was related to shelf life of the bananas (Lamikanra, 2002).

Peeling Method:

The type of peeling method used influence the degree of physiological response by tissues. In a study with carrots (Lamikanra, 2002) hand peeling, coarse and fine abrasion peeling was compared. Fine abrasion peeling resulted in lower weight loss of packaged slices as compared with coarse abrasion and the carrots hand-peeled with a sharp blade exhibited lower water loss, respiration and microbial counts than sliced carrots made from either fine or coarse abrasion.

There are several peeling methods available, but on an industrial scale, peeling is normally done mechanically using rotating carborundum drums, chemically using lye, or with high-pressure steam peelers (Alzamora et al., 2000).

Fresh cut citrus has shown more technical difficulties in peeling the fruit. The USDA and the FDOC have developed a process using enzyme infiltration under vacuum to facilitate citrus peeling. But the problem is that enzymes (cellulase and/or pectinase) continue their lytic action on the slices after the peeling process thus degrading the product quality during storage. There have been studies to use different solutions to stop enzymatic activity after peeling of Valencia oranges (Pinnavaia et al., 2005).

Sharp versus dull blades

The work by USDA researchers Bolin and Huxsoll on Shredded Lettuce (Bolin and Huxsoll 1991) is an example of the effect of sharp and dull blades on shelf life.

An example in fruits is the study by Portela and Cantwell (2001). They found that cutting cantaloupe melon pieces with a sharp borer resulted in longer shelf life at 5 °C than cutting with a blunt borer. Translucency is a common visual defect in commercial fresh-cut melon. Although the respiration and ethylene production rates were affected only slightly by cutting treatment, blunt cut pieces had higher ethanol concentrations, higher off-odor scores and higher electrolyte leakage than sharp cut pieces.

The reduction of mechanical injury will result in keeping better quality attributes of fresh cut by minimizing the number of injured cells whereas a blunt cutting instrument can induce injury to cells many layers removed from the actual cut because of the mechanical shock imparted to the tissue (Cantwell, 1998).

Temperature

The control of temperature is the most useful and important technique available for minimizing the effects of wounding in fresh cut fruit. (Brecht, 1995)

Low temperatures are needed to reduce respiration rates, retard microbial growth and retard deterioration processes such as softening and browning. In general, fresh cut product should be stored at 0 °C to 5 °C to maintain quality. For chilling-sensitive fruits, in general, low

temperatures retard the rate of deterioration of the fresh cut products more than they induce chilling injury (Kader, 2002).

O' Connor et al. (1994) in a study of the shelf life of minimally processed honeydew, kiwifruit, papaya, pineapple and cantaloupe compared the shelf life of fresh cut fruits storage at the temperatures recommended for whole fruit but determined that fresh cut fruit had longer shelf life at 4 °C than at the whole fruit recommended temperature when these were greater than 4 °C.

Watada and Qi (1999) estimated that 40% of fresh produce in the market is chilling sensitive. But due to the fact that fresh cut product are held only for a short period of time and are highly perishable when compared with the whole product, a temperature which causes a slight amount of chilling injury was preferred over a temperature which causes rapid deterioration. Cantwell et al. (1998) agreed with these findings, but indicated that is important that intact chilling sensitive commodities not be stored below their recommended temperature before they are prepared as a fresh cut product.

Many of the product quality, safety and packaging issues would be resolved if the chilled food distribution system or "Cold Chain" could be maintained steady between 33-40 °F. Regularly the cold chain temperature surpasses the 40 °F and typically is between 45 to 55 °F. Besides minimizing temperature changes, it is important to begin the cold chain as early a possible with hydrocooling, forced air-cooling or other method at the field level (IFPA, 2004).

There are many links in this chain, and many issues can happen throughout the chain. Processors, retailers, foodservice personnel and transportation companies must work together to ensure the cold chain for fresh-cut produce is properly maintained from beginning to end. But the problems of the cold chain remains at the points in the supply chain where product is transferred from one point to another such as from processor to warehouse, warehouse to truck and truck to final destination. This is where extreme temperatures outside of the acceptable 33° F to 40° F temperature range for fresh-cut produce can occur (Warren, 2005).

Related to the effect of temperature abuse, Gorny et al. (1998) reported that the shelf life of fresh cut nectarines and pears stored at 0 °C was reduced by half when the temperature was increased to 10 °C and this was attributed to higher respiration rates and ethylene production at higher temperatures. Rivera-Lopez et al. (2005) compared fresh cut papaya at 5, 10, 20 °C and determined that the fresh cut papaya stored at 20 °C showed the lowest total soluble solids and

the highest weight losses. After 6 days the losses in vitamin C were 5% at 10 °C and 63% at 20 °C but no losses were reported at 5 °C.

Temperature is a very important factor because when increases from 0 to 10 °C, respiration rate increases substantially with the Q_{10} ranging from 3.4 to 8.3 among various fresh-cut products and when temperature increases the deterioration increases (Watada and Qi, 1999).

Atmospheric composition

Low oxygen concentration and high carbon dioxide concentration are known to inhibit ethylene action and suppress respiration rate. The sensitivity of respiration to elevated carbon dioxide and reduced oxygen level depends on the commodity (Brecht, 1995).

The modification of the gas composition within fresh cut containers or bags can be beneficial in maintaining the quality of the fresh cut product. The mix of gasses was based on that recommended for the whole commodity. Although fresh cut products probably can tolerate more extreme levels of O_2 and CO_2 since they do not have the cuticle or skin to restrict gas diffusion and the distance of gas diffusion from the center to outside of the product is smaller than that for the whole commodity, the threshold level that can cause injury should be avoided because the gas mixture in the fresh cut packages cannot be regulated closely (Watada and Qi, 1999). The recommended modified atmospheres for different fresh cut fruits are summarized in Table 3-1.

Packaging technology is indispensable for most fresh cut products to be able to achieve a balance between the O_2 demand of the product for respiration and the permeability of the film to O_2 and CO_2 . Other factors need to be considered too, such as the specific cut, the quantity of product and the desirable equilibrium concentrations (Kader, 2002).

There are many examples of the benefit of modified atmospheric composition for fresh cut fruits discussed in Chapter 6. However, Figure 3-1 shows an example of the effect of atmosphere modification on peach slices by Gorny et al. (1999). Considering that whole peaches are tolerant to low O_2 and elevated CO_2 , the effect on peach slices was studied. The response of peach slices to low O_2 (0.25 kPa) and/or elevated CO_2 (10 or 20 kPa) atmospheres extended the shelf life of O-Henry peach slices kept at 10 °C by 1 to 2 days. The low O_2 and or elevated CO_2

concentrations reduced peach slice ethylene production and respiration rates. The problem was that production of fermentative metabolites (ethanol and acetaldehyde) in Elegant Lady peach slices was induced, which could cause objectionable off-flavors and odors. These results show the necessity of conducting sensory analysis since some atmospheres may extend shelf life based on visual quality but impart off-flavors.

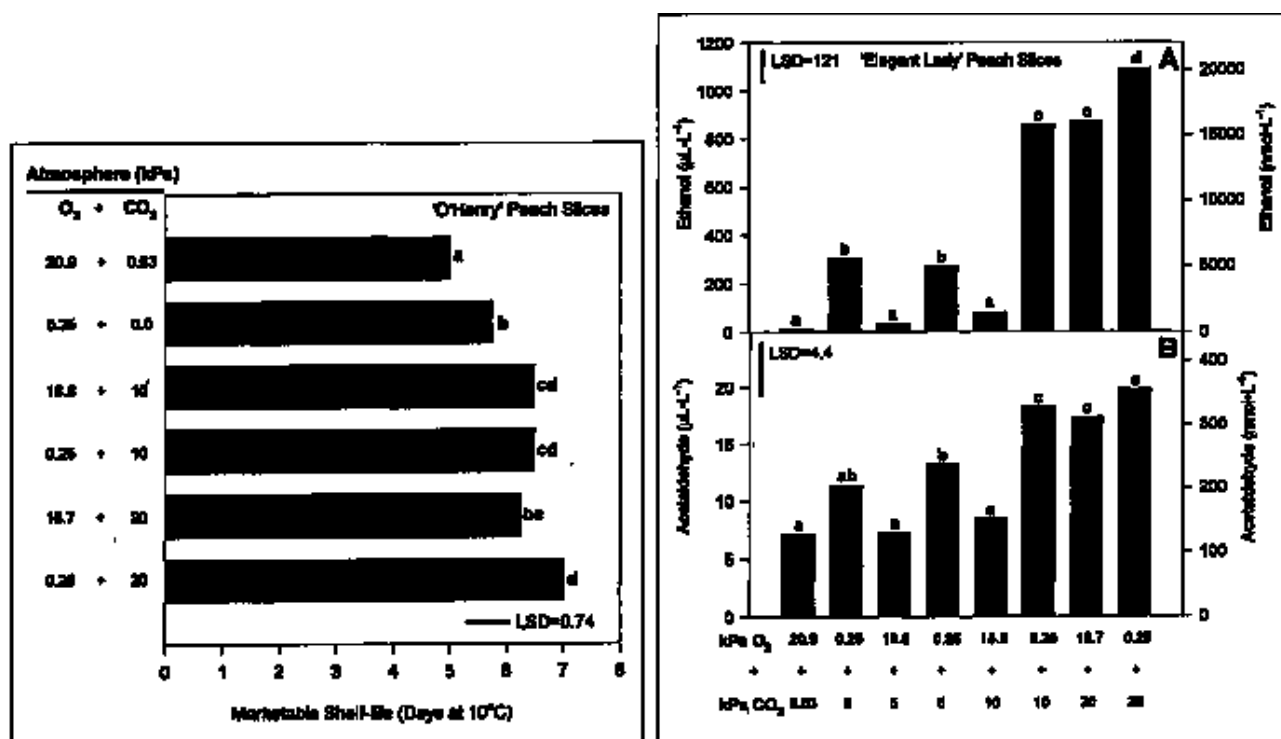


Figure 3-1 Peach slices stored at 10 °C under different atmospheres (Gorny et al., 1999)

One of the greatest benefits from the use of modified atmospheres is to be able to keep high relative humidity around the fresh cut product and because the natural barrier to water loss has been already removed this can reduce dehydration. However, water condensation on the inside of the package may promote the growth of spoilage microorganisms, but this can be minimized by appropriate selection of the film water vapor transmission rate or use of antifog additives into the film. The generation of a low O₂ and/or elevated CO₂ can slow the browning reactions, reduce the rate of product respiration, and reduce the C₂H₄ biosynthesis and its action (Gorny, 1997).

**Table 3-1 Recommended modified atmosphere concentrations for different fresh cut fruits
(Barta et al., 2006)**

| Commodity | Atmosphere |
|------------------|---|
| Apple | <1kPa O ₂ |
| Pear | 0.5 kPa O ₂ 2 kPa O ₂ |
| Peach | 2kPa O ₂ +12kPa CO ₂ 0.25 kPa O ₂ +10 kPa CO ₂ |
| Kiwifruit | 2 kPa O ₂ +5 kPa CO ₂ |
| Cantaloupe melon | 4 kPa O ₂ +10 kPa CO ₂ |
| Honeydew melon | 2 kPa O ₂ +10 kPa CO ₂ |
| Watermelon | 3 kPa O ₂ + 15 kPa CO ₂ |
| Mango | 2 kPa O ₂ +10 kPa CO ₂ |
| Persimmon | 2 kPa O ₂ +12 kPa CO ₂ |
| Strawberries | 1-2 kPa O ₂ + 10 kPa CO ₂ |
| Citrus | Air |

CHAPTER 4 - Biochemical consequences of wounding by cutting

Enzymatic effects

In the process of cutting the fresh cut product, the natural protection of the epidermis is removed and the cellular separation between enzymes and substrates are destroyed. These changes allow the enzymes and substrates to mix and react and cause changes in the tissues. The first change is the desiccation on the cut surface and then the enzymes and substrates are mixed causing sensory deterioration such as flavor and discoloration changes and loss of firmness. Some of the most important enzymes are discussed here (Lamikanra, 2002).

Lipoxygenase

Lipoxygenase is present in most plant tissues and catalyzes the oxidation of polyunsaturated fatty acids (containing a cis,cis 1,4 pentadiene structure) in the presence of oxygen. There are different types of lipoxygenases with different characteristics. While all three types of lipoxygenases could be present in plants such as legume seeds, some types are more dominant in others. The increase of lipoxygenase activity is related to plant tissue senescence. Inhibition of the lipoxygenase activity has been studied to delay ripening and softening in peaches and kiwifruit, it has been correlated with plant tissue development and pathogen and insect resistance mechanisms. Lipoxygenase, a hydroperoxide lyase is involved in the formation of volatiles from fatty acid precursors. The pathway of this enzyme is also responsible for production of C₆ aroma compounds in green leafy and fruit tissue (Brecht, J., 1995 and Lamikanra, 2002).

Karakurt and Huber (2002) compared intact and fresh cut papaya stored at 5 °C and studied the changes in firmness, cell-wall polyuronides (which are polymer of uronic acid such as pectin) and the activities of cell-wall and membrane hydrolases and ethylene biosynthetic enzymes (ACC:1-aminocyclopropane carboxylic acid enzymes). Firmness and molecular mass of polyuronides decreased more rapidly in fresh cut fruit than intact fruit. The activities of polygalacturonase, beta-galactosidases, lipoxygenase, phospholipase D, and ACC synthase and

ACC oxidase increased within 24 hours in fresh cut and were higher compared with the levels of intact fruit throughout the 8 days of storage. Pectin methyl esterase and phospholipase C activity showed no consistent differences between intact and fresh cut fruit. Overall, the data indicated that the wounding by cutting increases enzymes targeting cell walls and membranes.

Peroxidase

Peroxidase is found in almost all living organisms and the main function is to control the level of peroxides generated in oxygenation reactions to avoid excessive formation of radicals, which are harmful to all living organisms. This enzyme is relatively high heat stable and because of this reason is used in the processing of fruit and vegetables as a marker enzyme (Alzamora, 2000).

One of the important functions of peroxidase is related to the role in indole acetic acid oxidation action, by which participates in growth regulation. Peroxidases are considered to be indices of ripening and senescence, but are thought to be important in a variety of plant defense responses against pathogens. Peroxidase contributes to enzymatic browning because of its affinity to accept a wide range of hydrogen donors (Lamikanra, 2002)

Oms-Oliu et al. (2007) studied the role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. The packages had variable O₂ and CO₂ concentrations (2.5 kPa O₂ + 7 kPa CO₂, 10 kPa O₂ + 7 kPa CO₂, 21 kPa O₂, 30 kPa O₂ and 70 kPa O₂). Peroxidase activity, vitamin C content and total phenolics were monitored for a period of 14 days at 4 °C. The radical scavenging activity of fresh-cut melon strongly increased after 9 days storage related to a synthesis of phenolic compounds, mainly under 2.5 kPa O₂ + 7 kPa CO₂ atmospheres. The low O₂ levels maintained better the vitamin C and phenolic content during the storage. However, stressful too-low O₂ and high CO₂ levels induced an important increase in peroxidase activity under 2.5 kPa O₂ + 7 kPa CO₂ atmosphere, which was related to changes of vitamin C throughout storage. Therefore the 70 kPa O₂ atmospheres prevented anaerobic conditions during storage of fresh-cut melon and thus reduced wounding stress and deteriorative changes related to high peroxidase activity in tissue.

Lamikanra and Watson (2007) studied the effect of fruit heat pre-treatment with water at 60 °C on fresh cut cantaloupe melon, with and without calcium lactate (1%). The fresh cut processing was done immediately after heat treatment and storage of the fruit at 4 °C for 24 hours. There was a reduced respiration for heat-treated fruit and a reduced lipase activity in heat treated fruit, whereas storage at 10 °C and only the fruit cut 24 hours after treatment had reduced peroxidase activity. The use of calcium lactate did not affect respiration and textural changes caused by heat treatment, which showed increased hardness. Lipase activity was higher in fruit heated in calcium solutions.

Lamikanra and Watson (2001) studied the effect of ascorbic acid on the peroxidase and polyphenoloxidase activities in fresh cut cantaloupe melon. The presence of ascorbic acid (1.25 and 2.5 mM) reduced the activity of peroxidase over 60% at the time of processing probably as a result of a lower oxidative stress on the fruit surface. However, the peroxidase activity of fruit dipped in 1.25mM ascorbic acid increased after 2 days of storage, unlike those dipped in the 2.5mM. This indicates a depletion of residual ascorbic acid. The presence of trace metal ions such as Mn^{2+} increased ascorbate reduction of peroxidase, but exposure to an increased amount of metal ions inhibited this effect.

Polyphenol Oxidase

Polyphenol oxidases are a group of copper protein complex enzymes that catalyze the oxidation of phenolic compounds to produce brown pigments to cut or damaged surfaces of fruits and vegetables (Lamikanra, 2002).

Polyphenol oxidase is found widely in fruits and vegetable and the activity is principally controlled or inhibited by heat inactivation, the use of sulfur dioxide or sulfites or by addition of organic acids to lower the pH of the tissue (Collins and Marangoni, 2000)

Polyphenol oxidase is the most important enzyme associated with discoloration and appearance loss of fresh cut products. The effect of various antibrowning agents has been studied and will be discussed later in Chapter 6. Figure 4-1 shows the polyphenol oxidase activity of

fresh-cut pineapples at 10 °C treated with ascorbic acid (AA), isoascorbic acid (IAA) and N-acetyl-cysteine (AC) (Gonzales-Aguilar et al., 2005).

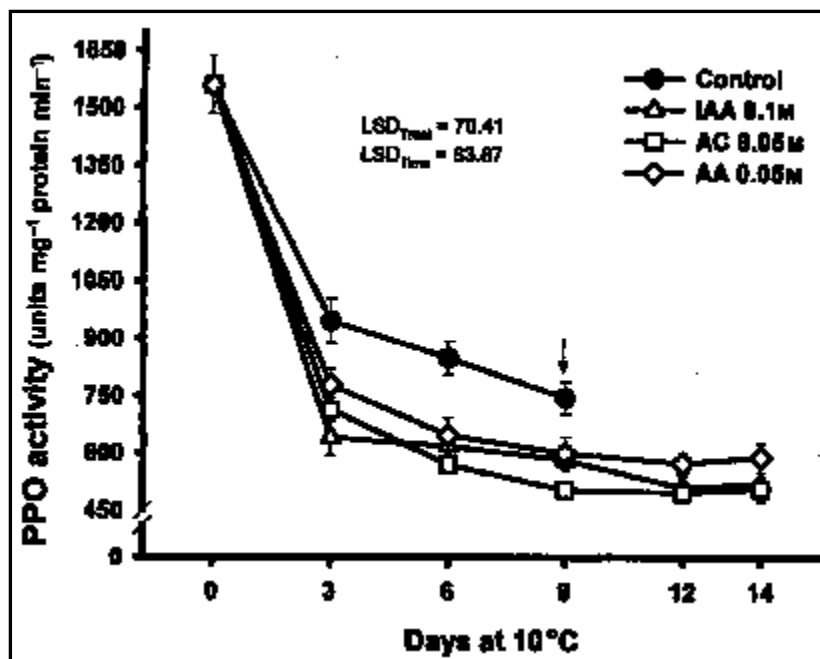


Figure 4-1 Polyphenol oxidase activity of fresh cut pineapples stored at 10 °C treated with isoascorbic acid (IAA), ascorbic acid (AA) , acetyl-cysteine (AC) (Gonzales-Aguilar et al, 2005)

The action of polyphenol oxidase results in the formation of highly reactive quinones that can then react with amino and sulfhydryl groups of proteins and enzymes as well as with other substances such as chlorogenic acid derivatives and flavonoids. All these reactions bring changes in physical, chemical, nutritional and sensory characteristics of fruits and vegetables (Lamikanra, 2002).

The activity of polyphenol oxidase was detected in all parts of the fruit, including the peel, flesh and cortex. Some studies in apples showed that the activity was higher in the peel and in the cortex (Alzamora, 2000).

Rocha and Morais (2001) studied the effect of controlled atmosphere (CA) storage on polyphenoloxidase (PPO) activity and phenolic content of fresh cut cubed apples (Jonagoed

variety). The atmosphere composition was 2% O₂ + 4% CO₂, 2%O₂ +8% CO₂, 2% O₂+12% CO₂ and the cubes were stored for 7 days at 4 °C. After 7 days, the samples stored in CA with concentrations of CO₂ higher than 4% had lower PPO activities than the air stored cubes. The treatment 2% O₂+12% CO₂ was found to be more efficient in reducing the color changes and inhibiting the polyphenoloxidase activity and although the total phenolic content was more variable this treatment showed the highest phenolic content because lower PPO activity may result in lower quantity of phenols degraded.

Song et al. (2007) showed that the content and proportion of the polyphenol varied depending on the variety of apples and that cider apples contained more polyphenol than juice apple varieties and that this is important as a reference for raw material selection, since total polyphenols content had a high correlation with browning.

Teixeira et al. (2007) studied fresh cut carambola, which is a fruit affected a lot by surface browning due to the activity of polyphenol oxidase. The slices were rinsed with NaOCl (20mg/L) drained and packed in PET (polyethylene terephthalate) trays, polystyrene trays covered with PVC 0.017mm or vacuum-sealed polyolefin bags and kept for 12 days at 6.8 °C and 90%RH. The results showed a lower PPO activity in the slices packaged in the vacuum-sealed bags, which kept better for up to 12 days.

Lamikanra and Watson (2001) in the study of the effect of ascorbic acid on peroxidase and determined the effect on polyphenoloxidase activities in fresh cut cantaloupe melon. The polyphenoloxidase was unaffected by ascorbic acid and the relative activity was very weak when compared to apple and lettuce. This low activity in cantaloupe and the absence of oxidizable phenolic compounds indicates that enzymatic browning reactions are not a contributing factor to the deterioration in cantaloupe.

Pectic enzymes

Pectic enzymes are important because their function and activity are related to the texture of fruits and vegetables and firmness retention is a very important quality parameter in fresh cut fruits.

Pectins are important components of the cell wall and middle lamella in higher plants. They are linear alpha-1,4 galacturonan chains with some esterified carboxyl groups, the amount of branching varies from one source to the other. Calcium is involved in forming intermolecular bridges by interaction with free carboxyl groups of pectin molecules. There are two main types of enzymes responsible for pectin degradation in fruits and vegetables. These are depolymerases (polygalacturonase and pectic lyase) and pectinesterase or also known as pectase (Alzamora, 2000). Figure 4-2 shows a fragment of a pectin molecule and the points of attack by the pectic enzymes (Lamikanra, 2002).

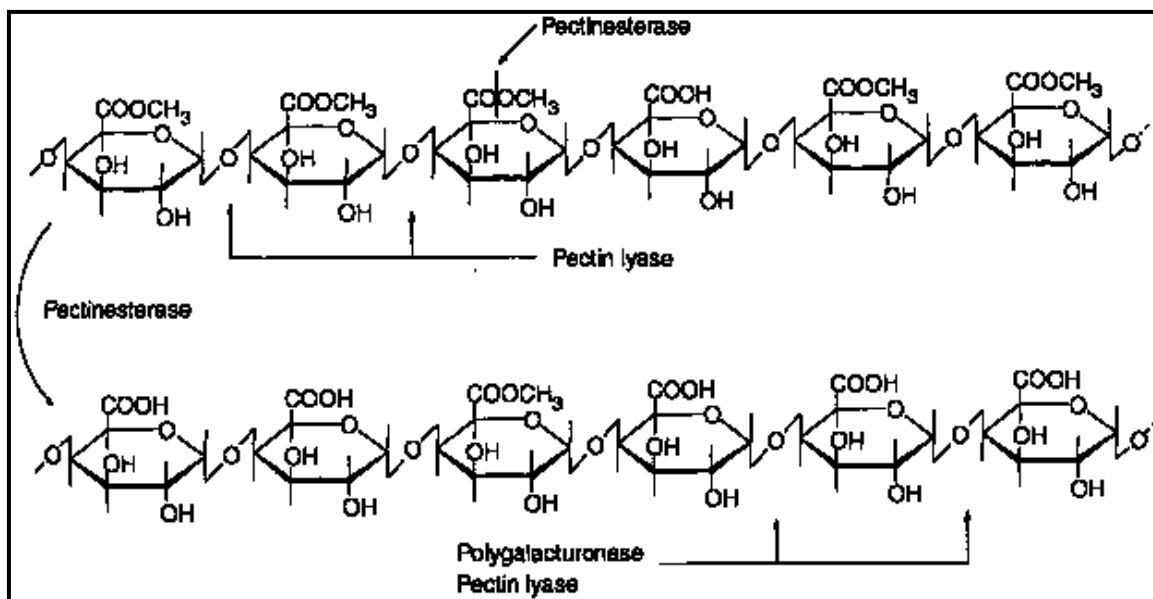


Figure 4-2 A Pectin fragment and points of attack by pectic enzymes (Lamikanra, 2002)

Polygalacturonase hydrolyzes glucosidic linkages and can be classified into endozymes that randomly cleave glycosidic bonds of pectin acids and polygalacturonates within the molecules at the alpha 1,4 linkages and exozymes that catalyze stepwise hydrolysis of

galacturonic acid from the nonreducing end of the chain. Some fruits that soften markedly during ripening such as pears and freestone peaches contain endo-polygalacturonase and exo-polygalacturonase. Other fruits such as apples and clingstone peaches contain only exo-polygalacturonase and showed slow softening characteristics (Camille, 2000 and Alzamora, 2000).

Many studies of the properties and characteristics of enzymes from the uncut produce and can serve as a base for fresh cut products, although there is a wide difference between fruits, cultivar maturity at harvest, harvesting and handling methods and storage conditions. For example, Goulao et al. (2007) monitored the activity of enzymes involved in fruit softening during ripening as resulting from the cell wall modifications. The enzymes monitored in the Gala apples were polygalacturonase (PG), pectin methylesterase (PME), pectate lyase (PL) and others. Exo-Polygalacturonase and pectate lyase activity increased in unripe fruit to fruit at harvest but were maintained at similar levels through the over-ripe stages. On the contrary, kiwifruit once harvested has an extended period during which most of the fruit softening occurs. Kiwifruit was harvested at 65 N firmness and was kept at 20 °C for 40 days until they reached the edible stage. Ethylene climacteric occurred when 10 N firmness was reached. Amylase activity was high at harvest and declined with a slight rise at 33 days. B-galactosidase activity was very low at the beginning of storage and increased throughout the experimental period, whereas polygalacturonase activity was detected only after the fruit was below 10 N. Endo 1,4 B glucanase decreased within the first 3 days and then increased and peaked 15 days after harvest; then remained low but increased again at the end of the storage period.

Lamikanra et al. (2003) determined the activity of polygalacturonase during storage of different cultivars of fresh cut cantaloupe and found that the enzyme activity increased for 3 days but then decreased for all cantaloupes cultivars (Figre 4-3). The enzymatic activity increased with storage temperature and varied for each cultivar. The rate of increase of polygalacturonase was unrelated to the fruit shelf life. However, the increase on its activity could be a result of conversion of the latent, inactive form of the enzyme or its precursor to the active enzyme in the cut fruit such as demonstrated with other fruits such as peaches. Bacterial production of polygalacturonase during storage could be a significant source of the increased activity.

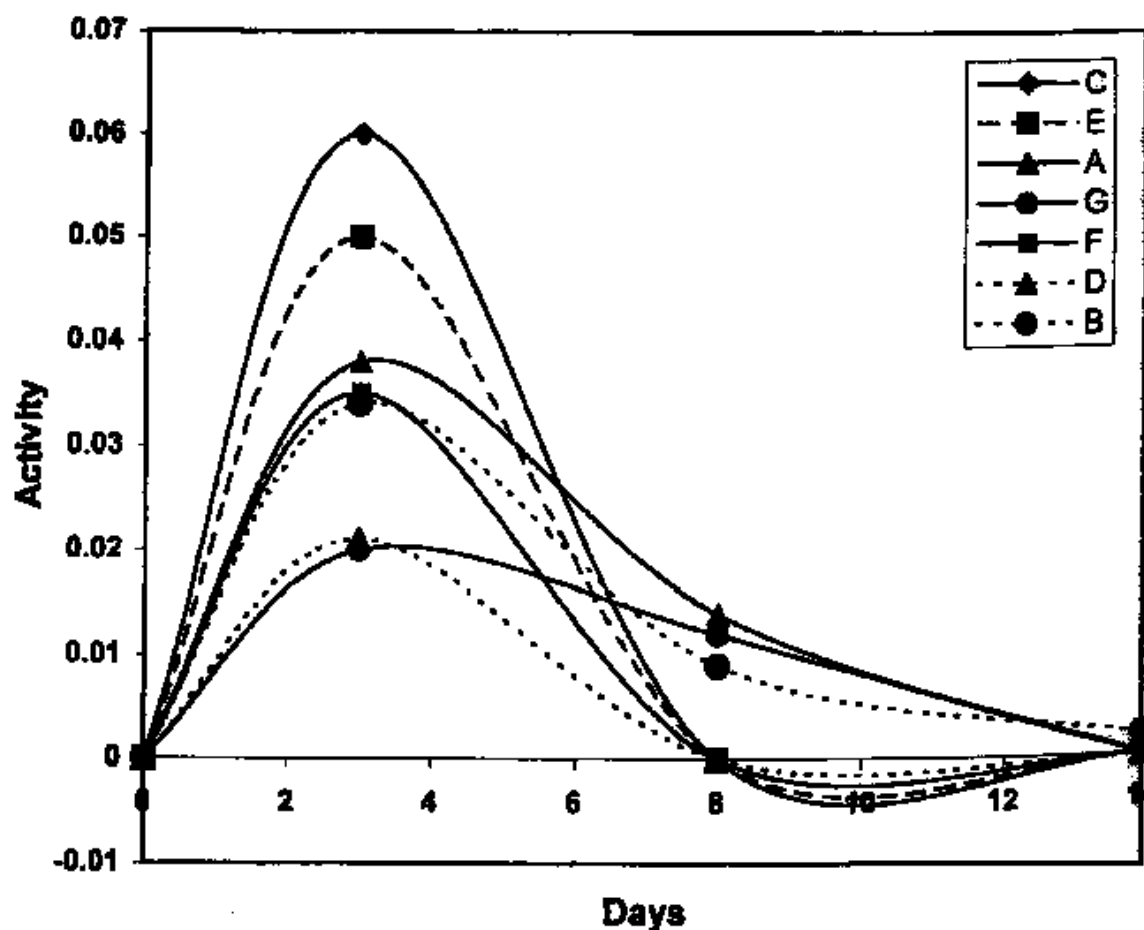


Figure 4-3 Effect of storage at 4 °C on polygalacturonase activity in fresh cut cantaloupe melon (A=Mission, B=S 1919, C=S3022, D=S1659, E=S1021, F=S10709, G=S2947 (Taken from Lamikanra et al., 2003)

Lyases cleave the alpha 1,4 galacturonosidic bond by trans elimination of hydrogen on carbon 5 of the galacturonic acid with the oxygen on the glycosidic bond. These enzymes are almost exclusively from microorganisms, although there are indications of their natural occurrence in some fruits (Lamikanra, 2002).

Pectinesterase catalyzes the demethylation of esterified pectin. Chung et al. (2006) studied the changes in the activity of the cell wall hydrolases: polygalacturonase, pectinesterase and B-galactosidase in wounded tomato fruit pericarp tissue. They concluded that in contrast to ripening fruit, wounding of fruit at the fully ripe stage appears to have no significant effects on the activities of any of enzymes studied.

CHAPTER 5 - Nutritional Changes in fresh cut Fruit

Nutritional value of Fruits

Fruits contain small to significant amounts of several important nutrients such as carbohydrates, vitamins and minerals, but other substances such as phytochemicals. Phytochemicals are defined as substances found in edible fruits or vegetables that exhibit a modulating effect in the metabolism when ingested (Camile, 2000). Table 5-1 shows some fruits and selected nutrients as a reference.

Table 5-1 Fruits and significant nutrients (Lamikanra et al., 2005)

| Substance | Source |
|----------------------------------|-------------------------------|
| Vitamin C | Citrus and other fruits |
| Potassium | Bananas and others |
| Fiber pectin and polysaccharides | Fruits in general |
| Alpha Beta carotens | Yellow/Orange fleshed fruits |
| Beta cryptoxanthin | Oranges and related fruits |
| Flavonoids | Apples, peaches, strawberries |
| Anthocyanins | Red/ purple Berries |

Besides the major macronutrients in fruits, there are some other compounds of fruits that can play a role in consumer preference in health. Some of these compounds influence the appearance, taste, color and aroma of fruits such as organic acids in fruits and their role in taste in conjunction with the sugar content or such as the esters of aliphatic alcohols and short chain fatty acids associated with the aroma. In addition to the flavor compounds there are pigments such as chlorophyll, carotenoids and anthocyanins responsible for the color of the fruits. Some of

these pigments have been shown to have a role in disease prevention. The phytochemicals previously mentioned are classified in eleven groups: carotenoids, dietary fiber, glucocinolates and others, inositol phosphates, polyphenols, phenols and cyclic compounds, phytoestrogens, plant sterols, protease inhibitors, saponins, sulfide and thiol containing compounds. Some of these phytochemicals are found in fruits such as citrus, papaya, pineapple, cherries, strawberries, peaches, apricots, watermelons and guavas (Cmille, 200 and Lamikanra et al., 2005).

Effect of Processing in the nutritional value

The nutritional quality of fruits may vary greatly according to cultivar and this nutritional status is an important factor in quality at harvest and postharvest life. The major pathways for potential loss are harvesting, storage, processing, storage and distribution. The objective of processing is to maintain the shelf-life, however processing might affect the nutrient content. These nutrient losses can be divided in three categories: intentional, accidental and inevitable. Intentional losses are the ones that occur due to removal of parts such as peeling; accidental losses are the ones that result from inadequate handling, and inevitable are the ones that occur in the heat labile nutrients destroyed by heat when blanching (Lamikanra et al, 2005).

Some of the common unit operations used in the preparation of fresh cut fruits and the effects on some nutrients are shown in Table 5-2.

Table 5-2 Effect of some fresh cut unit operations on selected nutrients (Adapted from Alzamora et al., 2000)

| Unit Operation | Effect on carotenoids | Effect on Anthocyanins | Effect on Phenolics |
|--------------------------------------|---|--|--|
| Peeling | - | - | Promotion of enzymatic browning |
| Size reduction | - | - | Promotion of enzymatic browning |
| Blanching | Beneficial when peroxidase is inactivated | Protection against coupled oxidation. Leaching when boiling water is used to blanch | Prevention of enzymatic browning |
| Acidification | Some xanthophylls transformation | Changes of pigment hue and chrome | Partial inhibition of PPO activity |
| Immersion in antibrownings solutions | Protection from oxidation | Leaching of soluble anthocyanins. Sulfites may cause discoloration | Protection from oxidation |
| Immersion in antimicrobial solutions | - | Leaching of soluble anthocyanins | Sorbates and benzoates may reduce enzymatic browning |
| Radiation | Gamma radiation has no effect in red capsicums and mangos | - | Promotion of browning in some cases |
| Modified atmosphere packaging | - | Destabilization in carbon dioxide | - |

Fresh Cut versus Fresh fruit: Nutrition

The demand for fresh cut fruit and vegetables has increased as well as the demand for products with as close as fresh-like quality as possible including the nutritional aspect. Fresh cut processing wounds the fruit tissues as described previously and there are many physiological disorders that affect the nutrient retention compared with whole fruits during storage.

In a study by Gil et al. (2006) the quality changes and nutrient retention in fresh-cut versus whole fruits during storage were evaluated and compared. This study was done with fresh cut pineapples, mangoes, cantaloupes, watermelons, strawberries and kiwifruits stored up to 9 days at 5 °C. Vitamin C losses after 6 days at 5 °C were $\leq 5\%$ in mango, strawberry and watermelon pieces, 10% in pineapple pieces, 12% in kiwifruit slices and 2% in cantaloupe cubes. There were no losses in carotenoids in kiwifruit slices and watermelons cubes, but losses in pineapple were the highest at 25% followed by 10-15% in cantaloupe, mango, and strawberry pieces after 6 days at 5 °C. Light exposure was a factor that promoted browning in pineapple pieces and decreased vitamin C content in kiwifruit slices. No significant losses in total phenolics were found in the fresh cut fruits after 6 days at 5 °C. The fresh cut fruits visually spoiled before significant nutrient loss occurred.

Figures 5-1 and 5-2 show the graphs that Gil et al (2006) obtained comparing the content of some nutrients for fresh fruit compared to fresh cut for pineapple, that had more losses, and for kiwifruit with fewer losses.

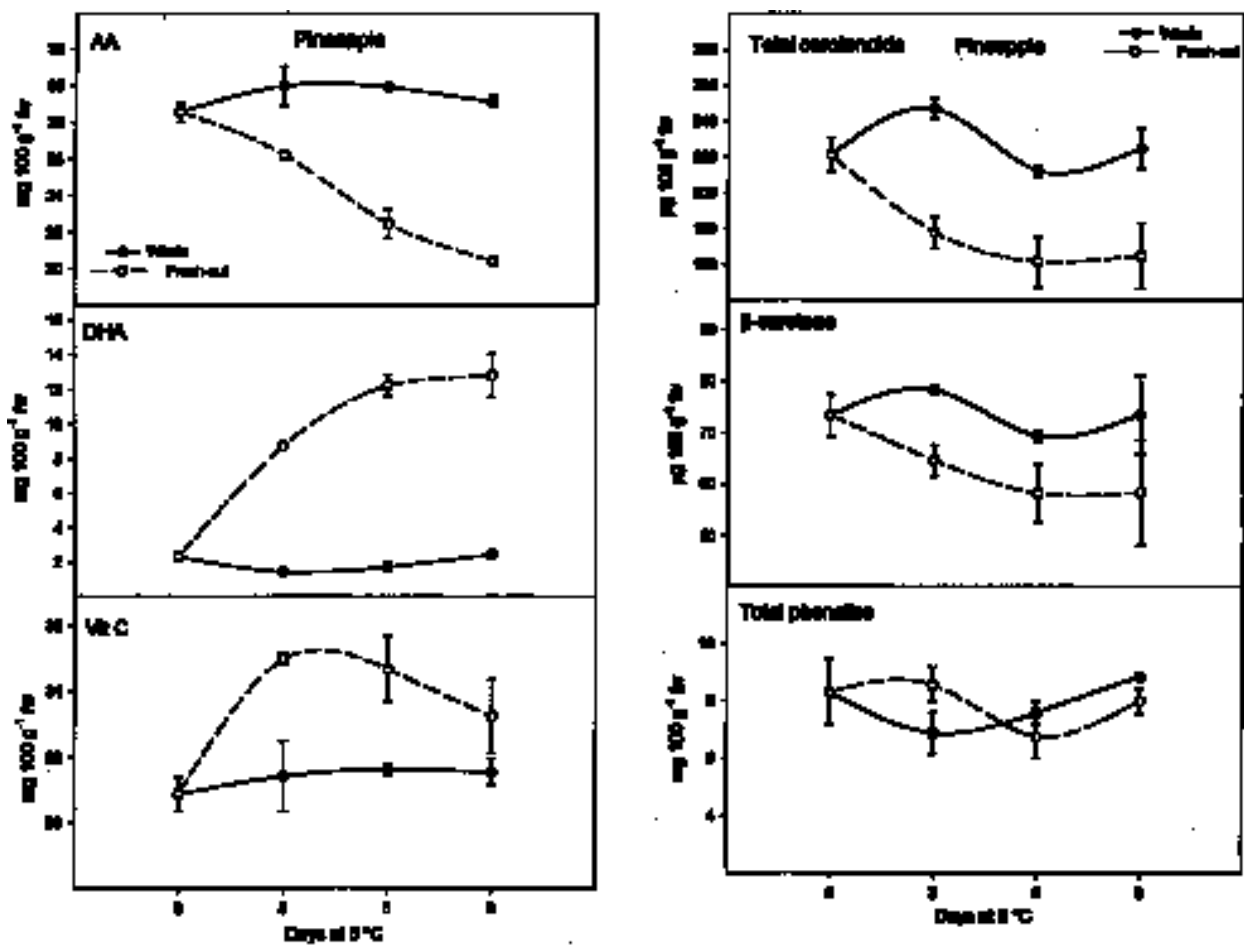


Figure 5-1: Nutrient retention in whole and fresh cut pineapple.

AA: Ascorbic acid, DHA: Dehydroascorbic acid, Vit C: Vitamin C. (Taken from Gil et al., 2006)

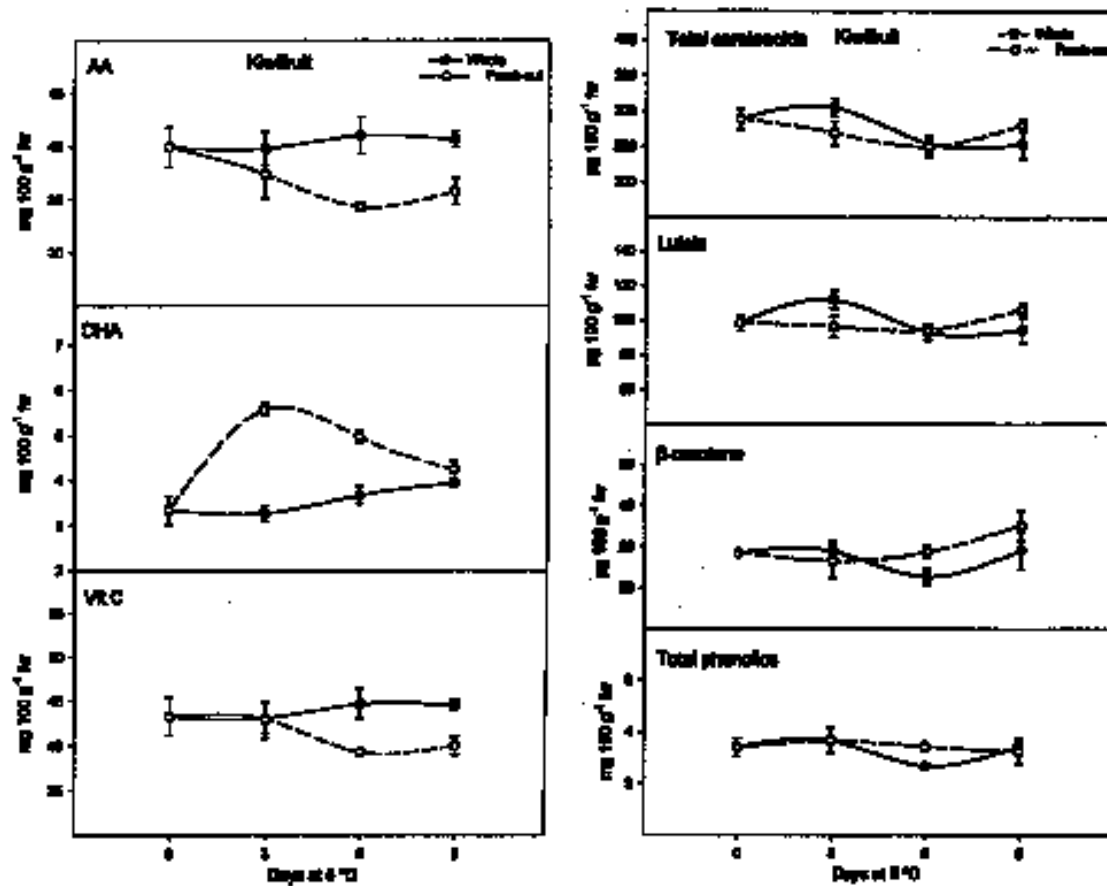


Figure 5-2: Nutrient retention in whole and fresh cut Kiwi Fruit.

AA: Ascorbic acid, DHA: Dehydroascorbic acid, Vit C: Vitamin C. (Taken from Gil et al., 2006)

Palmer and Kader (1997) determined the changes in quality and retinal equivalents (RE) and carotenoids in fresh cut peaches held for 7 days and persimmons held for 8 days at 5 °C in air or controlled atmospheres. Fresh cut peach slices stored in air and 12% carbon dioxide had a lower content of B-carotene and B-criptoxanthin thus resulting in lower RE than other treatments. Persimmons resulted in lower RE after 8 days in atmospheres of 2% oxygen or air plus 12% carbon dioxide. Although there were losses for peaches and persimmons, the losses of carotenoids were not significant as the limit of shelf life was reached before.

Red fleshed watermelons are a good source of the phytochemical lycopene. Perkins and Collins (2004) studied the lycopene stability of fresh cut watermelon of two cultivars stored at 2 °C for 2, 7 or 10 days. Lycopene content decreased 6 and 11% after 7 days of storage for each cultivar respectively.

Cocci et al. (2006) studied the effect of antioxidant dipping treatment (1% ascorbic acid and 1% citric acid for 3 minutes) and modified atmosphere in fresh cut apples. As a result of the antibrowning treatment the ascorbic acid treated samples had about 20-fold ascorbic acid higher than non treated samples at the beginning of storage and remained higher until the sixth day of refrigeration; total polyphenols were also higher for treated samples compared to those not treated. Results showed that the treatment used served the antibrowning purpose and in addition compensated the losses of the nutritional properties.

Another option to overcome the nutritional losses is to use the preserving treatment in fresh cut fruits to increase the nutritional content by the use of edible coatings and vacuum impregnation (treatments discussed in Chapter 6). Zao et al. (2004) used chitosan based edible coatings to increase the calcium and vitamin E of strawberries. Park et al. (2005) used vacuum impregnation for extending the shelf life but also to develop nutritionally fortified fresh-cut apples with vitamin E, calcium and zinc

Cisneros-Zevallos (2003) proposed a concept of applying postharvest abiotic stresses to enhance the nutraceutical content of fresh fruits and vegetables. Abiotic stress treatments are such as phytohormones, temperature, ultraviolet light, heat shock, water stress, wounding, etc.. The concept is that controlled stresses could be used to enhance the health benefits of fresh cut or even whole produce and adding more value to them.

CHAPTER 6 - Treatments to control changes in Fresh cut Fruits

Acidulants

The optimum pH for polyphenoloxidase has been reported to be from acid to neutral in most fruits and vegetables, and the optimum activity is observed at pH 6.0-6.5 and minimum activity is detected below pH 4.5. This is the reason behind the use of chemicals that lower the product pH or acidulants to help control the enzymatic browning. Acidulants are used in combination with other treatments because reducing browning by only controlling the pH is difficult. Acidulants such as citric, malic, and phosphoric acids are capable of lowering the pH of a system thus reducing the polyphenol oxidase activity (Lamikanra, 2002 and Marshall et al., 2000).

Citric acid is widely used as an acidulant and is typically applied at levels ranging between 0.5 and 2 percent (w/v) for the prevention of browning in fruits and vegetables. Citric acid can be used in combination with other antibrowning agents such as ascorbic or erythorbic acids and their neutral salts, for the chelation of prooxidants and for the inactivation of polyphenol oxidase. Besides lowering the pH, citric acid acts by chelating the copper at the active site of the enzyme (Marshall et al., 2000).

De Souza et al. (2006) used treatments of citric acid, calcium chloride, and reduced oxygen (2.5%) or high carbon dioxide (5-40%) atmospheres for mango (Kensington) stored at 3 °C. They concluded that the use of citric acid had little positive effect and appeared to promote softening. The best treatment was low oxygen and calcium chloride, that had a shelf life of 15 days.

Reducing Agents

Reducing agents react with quinones, reducing them to phenols and act on the enzyme polyphenol oxidase by linking irreversibly the copper of the enzyme. Reducing compounds are very effective in the control of browning (Lamikanra, 2002 and Marshall et al., 2000).

One of the most widely used antibrowning agents is ascorbic acid. Ascorbic acid is a moderate reducing compound, acidic in nature, forms neutral salts with bases and is water-soluble. Erythorbic acid, which is the D isomer of ascorbic acid but without the vitamin C activity, is cheaper than vitamin C and is believed to have the same antioxidant properties (Alzamora, 2000).

Sulfites are inhibitors of enzymatic browning. These compounds include sulphur dioxide (SO_2) and several forms of inorganic sulfites that liberate SO_2 . Although they are very effective, FDA has restricted their use due to potential allergic reactions (FDA, 2000 (b)). Lozano de Gonzales et al. (1993) compared the use of other antibrowning agents to sulphites showing sulphites were very effective compared to the rest, including ascorbic acid (Figure 6-1). The use of pineapple juice shown in this graph is discussed in the section of “Other antibrownings”.

Ascorbic acid reduces polyphenoloxidase browning by reducing o-quinones back to phenolic compounds before they form brown pigments. However, ascorbic acid is consumed in the process, providing only temporary protection unless used at higher concentrations. Gorny et al. (1999) determined that 2% ascorbic acid with 1% calcium lactate reduced the browning of fresh cut peaches initially but after 8 days at 0 °C the difference was minimal. Gil et al. (1998) determined that 2% ascorbic acid was effective in reducing the browning of fresh cut Fuji apple slices but in combination with low oxygen atmospheres storage.

Another reducing agent is cysteine, but for complete browning control, the amount of cysteine required is often incompatible with product taste (Lamikanra, 2002). The thiol-containing compounds such as N-acetyl L-cysteine and reduced glutathione are natural chemicals that react with quinones formed during the initial phase of enzymatic browning reactions. Oms-Oliu et al. (2006) used combinations of N-Acetyl-L-cysteine, reduced glutathione, ascorbic acid and 4-Hexylresorcinol and concluded that 0.75% of N-Acetyl-L-cysteine was effective to prevent browning of fresh cut pears up to 28 days at 4 °C and 0.7% glutathione was effective up to 21 days at 4 °C. There was also an enhanced effect combining N-

Acetyl-L-cysteine with reduced glutathione. Ascorbic acid and 4-hexyresorcinol were not effective.

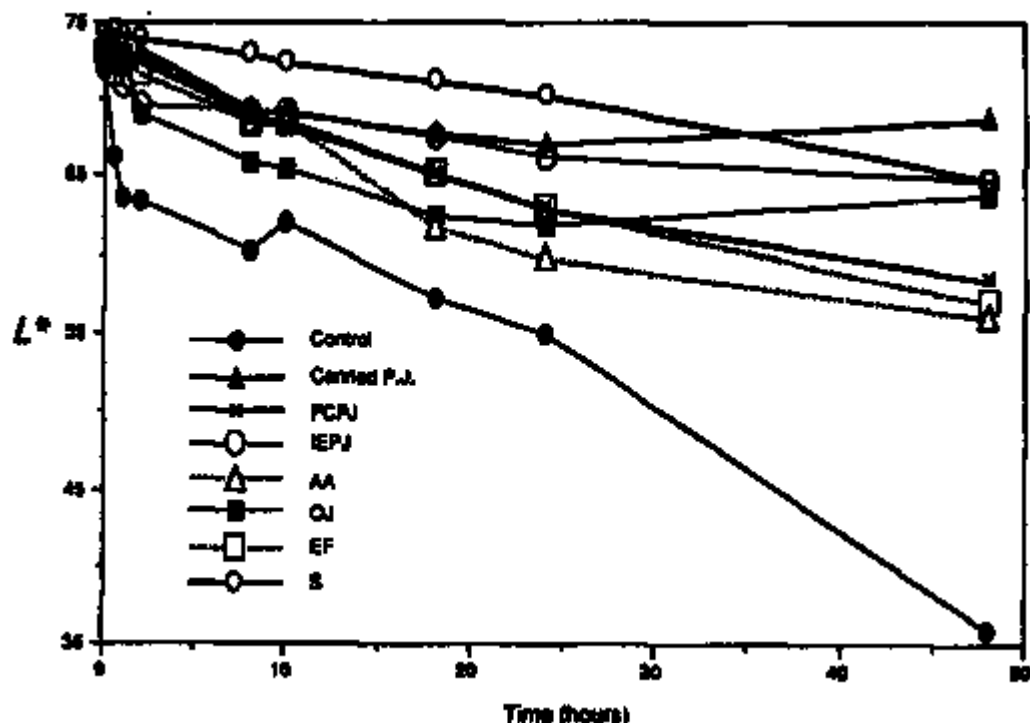


Figure 6-1: Change in L value (Lightness) in fresh apple rings stored at 21 °C. Treatments: Control (water), PJ (12.8 Brix pineapple juice), FCPJ (12.8 Brix Frozen concentrate pineapple juice), IEPJ (12.8 Brix Ion exchanged canned pineapple juice), AA (0.7% Ascorbic acid), OJ (11.8 Brix frozen concentrated orange juice, EF (commercial solution of Ever Fresh), and Sulfite (0.1% sodium bisulfite)

Rojas-Grau et al. (2006) compared the browning inhibition of N-acetyl cysteine, glutathione, ascorbic acid and 4-Hexylresorcinol with “Fuji” apples stored for 14 days at 4 °C. They determined that the best concentrations were at least 0.75% of N-acetylcysteine, 0.60% of N-acetylcysteine with 0.60% of glutathione were the best treatments. The sensory effects of the treatments were not determined.

Gonzalez-Aguilar et al. (2005) compared N-Acetyl cysteine with ascorbic acid and isoascorbic acid as antibrowning agents for fresh cut pineapple stored for 14 days at 10 °C.

While the treatment with N-acetyl-cysteine (0.05M) was the most effective in reducing browning and better appearance, higher levels of sugars and vitamin C (0.05M) resulted from isoascorbic acid (0.1M) and ascorbic acid. The level of antibrowning used did not affect other sensory characteristics.

Chelating agents

Chelating agents prevent enzymatic browning through the formation of a complex between these inhibitors and copper through an unshared pair of electrons in their molecular structures. Some of the chelating agents use on fruits and vegetables are citric acid and EDTA (ethylenediamine tetraacetic acid) (Alzamora, 2000). EDTA is used with other antibrowning chemicals in concentrations up to 500ppm (Lamikanra, 2002). Some tests using EDTA as an inhibitor of peach polyphenol oxidase were not totally effective (Marshall et al., 2000).

Gardner et al. (1990) filed a patent for Sporix, which is an acidic polyphosphate, a powerful chelator and acidulant. This patent refers to the process and sulfite-free solution to preserve fresh-peeled vegetable and fruits, as well as fresh leafy vegetables. Fresh peeled fruits or vegetables are preserved by a dipping process or by spraying a mix of the commercial preservative Sporix and citric acid. After trimming, the peeled vegetables or fruits are subjected to a second exposure of the same solution; and then packed and refrigerated for up to 12 days without suffering discoloration or spoilage. Sporix was used in cut surfaces in combination with ascorbic acid, although not approved in the U.S. for food use (Lamikanra, 2002).

Phosphates have been used as components of commercial browning inhibitors. Pilizota and Sapers (2004) used combinations of sodium hexametaphosphate, ascorbic acid, calcium chloride, sodium chloride, and sodium erythorbate with different levels of citric acid to adjust the pH to develop an acidic browning inhibitor to target the core browning of fresh cut apples slices but without affecting the tissue by the lower pH. The best treatments were 3% ascorbic acid +1% citric acid+1% sodium hexametaphosphate that had a pH of 2.9, but the problem was that in some cases sodium hexamethaphosphate induced tissue breakdown with both varieties tested but only at 10 °C. Although no formal sensory evaluation was done some sour flavor was detected.

Kojic acid is 5-hydroxy-2-hydroxymethyl-4H-pyran-4-one, a γ -pyrone derivative and a fungal metabolite produced by many species of *Aspergillus* and *Penicillium* and a good metal ion chelator (Marshall et al., 2000). Son et al. (2001) used kojic acid among other thirty six antibrowning compounds to compare the inhibitory effect on apple slices. Kojic acid, oxalic acid, oxalacetic acid, ascorbic acid, cysteine, glutathione, N-acetylcysteine and 4-hexyl resorcinol were grouped as the ones to show the highest inhibitory activity on apple browning. The minimal concentrations for an effective antibrowning activity were 0.25 % oxalacetic acid, 0.05% oxalic acid, 0.05% cysteine and 0.05% kojic acid.

Inorganic salts

Salts of calcium, zinc, and sodium have been tested as antibrowning agents that act by inhibiting the enzyme polyphenol oxidase. However, chloride is a weak inhibitor; some authors report that the chloride levels required to inhibit the enzyme are too high and compromise the taste (Lamikanra, 2002). Other studies tested a mix of ascorbic acid-sodium chloride, which inhibit 90-100% of the polyphenol oxidase activity (Alzamora, 2000).

Lu et al. (2007) used sodium chlorite in fresh cut apple slices in dipping treatments solutions for 1 minute, drained the slices, placed them in plastic containers at 20 °C for 24 hours and then stored them in polyethylene bags at 5 °C for 2 weeks. The treatments were sodium chlorite, sodium chlorite acidified with organic acids, and other salts. Apple slices treated in acidified sodium chlorite or sodium chlorite alone had a significantly smaller decrease in lightness value (L^*) indicating less browning than those treated in citric acid or water control at 4 hours. After 2 weeks of storage, only sodium chlorite (0.5–1.0 g/L), sodium bisulfite (0.5 g/L) and calcium l-ascorbate (10 g/L) continued to inhibit browning. Treatment with 0.5 g/L sodium chlorite and pH adjusted in the range from 3.9 to 6.2 using citric acid reduced browning more effectively than 0.5 g/L sodium chlorite without pH adjustment. Two organic acids, salicylic acid and cinnamic acid, when added to sodium chlorite solution, were found to achieve even better inhibition of browning than citric acid at the same pH value.

Complexing agents

These are agents that entrap or form complexes with the substrates of the enzyme polyphenoloxidase or to reaction products. Some of the complexing agents are cyclodextrins of cyclic nonreducing oligosaccharides of six or more d-glucose residues. The problem that some researchers have observed is that β -cyclodextrin has low water solubility and in some experiments with apples was not effective or not consistent in controlling browning (Lamikanra, 2002).

Lopez-Nicolas et al. (2007) used different types of cyclodextrins as secondary antibrowning agents in apple juice and determined that maltosyl- β -cyclodextrin can enhance the ability of ascorbic acid to prevent the enzymatic browning due to the protective effect against ascorbic acid oxidation. Alvarez-Parilla et al. (2007) compared the polyphenol oxidase inhibitory effect of β -cyclodextrin, 4-hexylresorcinol and methyl jasmonate in red delicious apple. The inhibitory strength was higher for 4-hexylresorcinol followed by β -cyclodextrin and then methyl jasmonate. There was also a dual synergistic effect between β -cyclodextrin and 4-hexylresorcinol.

Chitosan, a naturally abundant polymer of b-(1-4)-N-acetyl-D-glucosamine, is derived from the chitin of shellfish. Chitosan has shown antimicrobial and antibrowning properties. Chitosan has shown effect in inhibiting postharvest pericarp browning of lychee fruits (Jiang et al., 2005). Chitosan was studied in the application of coatings to control browning in fresh-cut Chinese water chestnut (Pen and Jiang, 2003) and as a coating to improve the quality of fresh cut guava (Thommohaway, 2007).

Enzyme inhibitors

4-hexylresorcinol is an antibrowning agent with potential for application to fresh cut products. It is a chemical used in medications and used to prevent the discoloration of shrimp (Lamikanra, 2002).

Dong et al. (2000) used 4-hexylresorcinol with a combination of other compounds to extend the shelf life of fresh cut Anjou pears. They determined that 4-hexylresorcinol (0.005 and

0.01%) was effective to prevent browning in combination with 0.5% ascorbic acid but there was no effect without ascorbic acid. Sensory evaluation indicated that 0.01% of 4-hexylresorcinol was detected by panelists.

Honey has been studied for its antioxidant capacity and is believed to contain a small peptide that inhibits the activity of polyphenoloxidase (Marshall et al, 200). Jeon and Zhao (2004) evaluated ten different honeys from floral sources and their antibrowning effect on fresh cut apples. The apples were vacuum impregnated in the 10% honey solutions and the color was monitored for 10 days during storage at 4 °C and 80% RH. The Wildflower and Alfalfa honeys significantly inhibited browning discoloration, although there was an initial reduction of lightness as a result of the color from honey. A honey with light color may be preferred to be used as an antibrowning agent for fresh cut apples.

Combination of antibrownings

Other studies with fresh cut mangoes used combinations of antibrowning agents and modified atmosphere packaging (MAP) and resulted in a reduction of browning and deterioration of fresh-cut mangoes stored at 10 °C. The combinations of several browning inhibitors may be more effective than individual ones. Among these treatments, solutions containing 4-hexylresorcinol (0.001 M) plus potassium sorbate (0.05 M), 4-hexylresorcinol, potassium sorbate and D-isoascorbic acid (0.5 M) reduced changes in color and microbial growth and did not affect sensory characteristics of fresh-cut mangoes (Gonzalez-Aguilar, 2000).

Mohammed and Wickham (2005) dipped pineapple slices in solutions containing 300ppm ascorbic acid or 200ppm 4-hexylresorcinol or 300ppm ascorbic acid + 200ppm 4-hexylresorcinol. The fresh cut pineapple was packed in a modified atmosphere (MAP) and stored up to 4 days at 10 °C. Separate treatments with ascorbic acid or 4-hexylresorcinol in conjunction with MAP controlled browning and maintained quality of fresh-cut pineapple slices for 2 days at 10 °C, but the combined treatment was more effective in browning inhibition and microbial spoilage over the longer storage period.

Gorny et al. (2002) determined that a treatment with 2% ascorbic acid, 1% calcium lactate and 0.5% cysteine adjusted to pH 7.0 inhibited loss of firmness and prevented browning in “Bartlett” pear slices in combination with low O₂ and elevated CO₂ atmospheres without producing objectionable off-flavors.

Other antibrownings

Enzymatic treatments with proteases that attack polyphenol oxidase have been suggested as alternative prevention treatments for enzymatic browning. Some preliminary tests used small pieces of apples and potatoes dipped for 5 minutes in a 2% enzyme solution and results showed that papain worked best on apples, whereas ficin (enzyme from figs) worked better on potatoes (Lamikanra et al., 2002). Forget et al. (1998) studied the antibrowning efficiency of papaine extracts by studying their activity through two mechanisms: polyphenol oxydase inactivation and presence of quinone trapping substances.

Concern by some consumers to avoid food preservatives has lead to some natural options. Lozano de Gonzales et al. (1993) used pineapple juice for antibrowning, considering that pineapple contains the enzyme bromelain, which also has properties inhibiting enzymatic browning as well as ascorbic acid. Pineapple juice was an effective browning inhibitor in both fresh and dried apples. All fractions of pineapple juice separated by different extractions methods inhibited enzymatic browning at least by 26% as measured colorimetrically and by visual examination. Fractioning identified that the inhibitor is a neutral compound of low molecular weight.

Song et al. (2007) used rhubarb juice as a natural antibrowning agent for fresh cut apples slices. They found that juices at 20% concentration containing 67mg/100 g of oxalic acid inhibited browning. Yoruk and Marshall (2003) investigated the mode of inhibition of oxalic acid on polyphenoloxidase and determined that by binding with copper to form an inactive complex, reduce the catechol-quinone product formation. Oxalic acid was a more potent inhibitor of polyphenoloxidase compared with other structurally related acids.

Other compounds such as benzoic and cinnamic acids are polyphenol oxidase inhibitors but have been found not to give prolonged protection over storage time (Lamikanra, 2002).

Roller and Seedhar (2002) used cinnamic acid to inhibit microbial growth in fresh cut melon and kiwifruit.

Hexanal is a natural aroma precursor in apples. Hexanal enhances aroma, but has been used to reduce enzymatic browning as well as to inhibit molds, yeasts and mesophilic and psychrotrophic bacteria in apples slices (Beaulieu and Gorny, not dated).

Li et al. (2007) used oxyresveratrol (*Morus alba* L. twig extracts used in Chinese traditional medicine) as an antibrowning agent for cloudy apple juices and fresh cut apples. They used 0.001 M oxyresveratrol, 0.5 M isoascorbic acid, 0.05 M calcium chloride and 0.025 M acetyl cysteine and did not observe any browning in apple slices after 28 days at 4 °C.

Calcium treatments

Calcium treatments are commonly used in the industry as firming agents for canned tomatoes, cucumber, other vegetables, and have been reported to reduce browning. Calcium dips have shown benefit for whole apples, peppers, strawberries, tomatoes, and peaches. The effect of calcium on texture can be explained by different mechanisms: 1. Complexing of calcium ions with cell wall and middle lamella pectin; 2. Stabilization of the cell membrane by calcium ions and 3. Effect of calcium on cell turgor pressure (Luna-Guzman and Barret, 2000). The firming action of calcium at the same time contributes to a reduced leakage of polyphenol oxidase and its substrates at the exposed cut surfaces contributing to reduce browning (Lamikanra et al., 2002).

Calcium chloride has been used as a firming agent, but the disadvantage is that may impart undesirable bitterness or flavor differences to the product. Calcium lactate is an alternative source of calcium (Luna-Guzman and Barret, 2000).

Combined treatments of low temperature blanching to activate the enzyme pectinesterase prior to the calcium dip have also been considered. The enzyme pectinesterase causes de-esterification of pectin and increases the number of calcium binding sites (Lamikanra, 2002).

Luna-Guzman et al. (1999) applied calcium chloride dips (1-5%) to melon cylinders. Production of CO₂ was higher in untreated samples than in calcium treated and intact fruit. Calcium chloride dips improved firmness of fresh cut cantaloupe stored at 5 °C. Firmness

improved with higher calcium chloride concentration and calcium concentration in the tissue of melons was increased by 300%

Luna-Guzman and Barret (2000) compared the effects of calcium chloride and calcium lactate in maintaining the quality of fresh cut cantaloupes. Fresh cut cantaloupe cylinders were dipped for 1 min in 2.5% solution of calcium chloride at 25 °C and calcium lactate at 25 °C. Microbiological and sensory characteristics were determined, as well as respiration and ethylene production during 12 days at 5 °C. Both salts kept melon firmness, but calcium chloride imparted bitterness, whereas for calcium lactate did not.

Ethylene Scrubbing

1-methylcyclopropene (1-MCP) has been shown to block ethylene action, inhibiting ethylene responses such as ripening and softening. Therefore could be useful for maintaining the quality fresh cut products (Lamikanra, 2002).

Vilas-Boas and Kader (2001) used a 6 hour exposure at 50 °F to 1000 ppb 1-MCP on several fresh cut fruits kept at 41 °F until the end of their marketable life. The MCP treatments were applied to the intact fruit before cutting and to the fresh cut products after cutting. The fruits tested were bananas, kiwifruits, mangoes and persimmons. The exposure to 1-MCP of bananas before cutting stimulated more ethylene production and did not influence softening or browning rates of banana slices. Exposure of the slices already cut had no effect on their ethylene production and browning rates, but reduced their softening rate and extended their post cutting life by 1 to 2 days at 50 °F. The application for whole kiwifruit delayed the softening of the slices by 1 to 2 days at 41 °F and ethylene production rate was decreased. The application for mangoes (Keitt) was not effective in slowing the softening rate of fresh cut mango cubes over 3 days at 41 °C. But treating fresh cut mango cubes with 1-MCP was effective in delaying their softening for 1 to 2 days at 41 °C. For whole persimmons, the treatment with 1-MCP was more effective in delaying softening than treating fresh-cut wedges, but it increased the ethylene production rate during storage at 41 °C and a 3 to 4 day extension of post cutting life at 41 °F.

Linchun et al. (2007) reported that the application of 1-MCP treatments before cutting had beneficial effect on reducing wound responses on kiwifruit stored at 2 °C for 10days. The

treatment with 1MCP resulted in reduced respiration rate, ethylene production, lowered electrolyte leakage, and delayed softening and color change.

Calderon-Lopez et al. (2005) studied the effect of 1-MCP on whole fruit and fresh cut apple slices to five cultivars of apples (Delicious, Empire, Idared, Law Rome and Mutsu). The response to 1-MCP was a function of cultivar. The treatments of apples at harvest with MCP maintained a fresh cut product with an extended shelf life. But in general, slices untreated and treated with 1-MCP deteriorated at similar rates.

Aguayo et al. (2006) determined the combined effect of 1-MCP, calcium chloride dip and atmospheric modification on the quality of fresh cut strawberries. The 1-MCP was applied before (whole product) or after cutting strawberries wedges and storage in air plus 1 $\mu\text{L/L}$ C_2H_4 for 24 hours at 5 °C. When 1-MCP was applied alone (before or after cutting) there was not significant effect on firmness and appearance during storage for 12 days at 5 °C in spite of the increased respiration rates but reduced C_2H_4 rate. The combined treatment of 1-MCP plus CaCl_2 plus control atmosphere (3 kPa O_2 + 10 kPa CO_2) reduced softening and deterioration rate and microbial growth with 9 days of shelf life, whereas the control had 6 days at 5 °C.

Heat treatments

The use of heat has been used in fruit and vegetables for several reasons such as control of fungal spores, insect infestation, inhibit ethylene synthesis, inhibit cell wall degradation associated with hydrolytic enzymes, demethylation of pectin by pectin methylesterase, and increased synthesis of heat shock proteins (Lamikanra et al., 2005).

Abreu et al. (2003) used mild heat pre-treatments on Rocha pear prior to cutting. The cut fruit was stored for 7 days at 2 °C. The best treatment to preserve color was 36-45 °C with treatment times greater than 40 minutes. Treatments used with temperature greater than 45 °C increased firmness from an initial value of 27.2 to 70 N. Heat pre-treatments produced an increase in pH values from 4.5 to 5.8 and to preserve the pH the best conditions were the ones with temperature lower than 45 °C for less than 150 minutes.

Lamikanra et al. (2005) determined the effect of mild heat pre-treatment for quality retention of fresh cut cantaloupe melon. Whole cantaloupes were treated at 50 °C for 60 minutes. After heat treatment, cantaloupes were stored at 4 °C before cutting for 24 hours and then stored at 10 °C for 8 days. Sensory evaluation indicated that the heat treatment increased desirable attributes described as fruity melon and sweet aromatic flavors and reduced total microbial counts and prevent growth of lactic acid bacteria. Heat-treated fresh cut melon had also lower respiration rates and reduced moisture loss during storage.

Lamikanra and Watson (2006) determined the effect of mild heat treatment in solutions with and without calcium lactate. Whole fruit that was held at 4 °C before treatment was immersed in hot water at 60 °C with and without dissolved calcium lactate (1%). There was an improvement in shelf life on heat pre-treated fruit, but the addition of calcium lactate did not improve product quality.

Solomon et al. (2006) treated cantaloupes with hot water, but in this case the cantaloupes were inoculated with Salmonella prior to be heat-treated. Treatments with water at 85 °C for 60 and 90 seconds resulted in reduction of up to 4.7 log colony-forming units per square centimeter of rind. The fruit treated at 85 °C for 90 seconds was softer than the ones treated for 60 seconds. The results on inactivation of salmonella have potential to be used by producers of fresh cut melon since also the heat penetration measured indicated that the edible portions of cantaloupes remained cool.

Kim et al. (1993) evaluated heat treatments at various times and temperatures on eleven apple cultivars. The different cultivars had different susceptibility to browning depending on heating temperature and heating time. The varieties Delicious and Golden Delicious showed the strongest tolerance to heat treatment. Overall, the apples treated at 45 °C produced slices with less browning with up to 90 minutes of treatment. Among cultivars heated at 45 °C, only slices of Golden Delicious, and Delicious were firmer than non-treated slices. Five cultivars (Empire, Golden Delicious, McIntosh, New York 674 and Delicious) were the treatments that showed relatively low incidence of browning and relatively firmer texture by heat treatment; these treatments were used to investigate the response for 8 days at 2 °C as fresh cut fruit using a heat treatment at 45 °C for 105 minutes. In most cases, the respiration rates of apple slices prepared from heated apples was slightly lower than those of the non-treated apples. All 5 cultivars had less browning and increased firmness than non-treated apples.

Modified atmosphere packaging

The principle for modified atmosphere packaging (MAP) technology involves placing the product in a sealed package and establishing different environment conditions inside the package. The most important aspect of modified atmosphere packaging is to determine the type of environment will be most beneficial for the product and the type of materials that can be used to create the optimal environment. The critical conditions are: atmospheric oxygen, carbon dioxide, ethylene and relative humidity. The shelf life will improve if all these conditions are chosen correctly, but incorrect conditions can reduce the shelf life (Gorny, 1997).

One benefit of MAP is that can be kept high relative humidity around the fresh cut product. Although water condensation can be a disadvantage, since it can promote the growth of spoilage microorganisms and the visibility of the product by the consumer. The selection of films involves the consideration of water vapor transmission rate and possible incorporation of antifog additives (Gorny, 1997). Other benefit of MAP is that low oxygen concentration or elevated carbon dioxide concentration inside the fresh cut MAP can slow browning reactions, reduce rate of product respiration and reduce ethylene biosynthesis and effects (Gorny, 1997).

Modified atmospheres can be achieved passively or actively. A passive MAP occurs when fresh produce is hermetically sealed in a semi permeable container and the respiration process alters the gas composition. An active MAP can be achieved by flushing out the air within the package with a precise mixture of gases to obtain an initial atmosphere (Lamikanra et al., 2002).

Martinez-Ferrer et al. (2002) reported the use of modified atmospheres for fresh cut mango and pineapple using a gas mixture of 4% oxygen, 10% carbon dioxide and 86% nitrogen. Microbial growth, texture and color were significantly different between the gas mixture and other treatments (100% oxygen, vacuum and control). The gas mixture (4% oxygen, 10% carbon dioxide and 86% nitrogen) was the most effective MAP system in extending the shelf life of the fruits. This treatment, in combination with blanching and ascorbic acid dipping controlled significantly the growth of spoilage microorganisms, particularly yeasts and molds, although the sensory analysis showed slight difference between fresh and MAP mango, there was no difference between fresh and MAP pineapple.

Marreno and Kader (2006) kept fresh cut pineapples at different storage temperatures and modified oxygen and carbon dioxide concentrations. The fresh cut life varied from 4 days at 10 °C to over 2 weeks at 0 °C. The advantage of the use of modified atmospheres (8% oxygen and 10% carbon dioxide) was a better retention of color.

Gorny et al. (2002) working with controlled atmospheres and chemical preservatives in fresh cut Barlett pears slices found that low oxygen (0.25-0.5 kPa) and elevated carbon dioxide concentration (5, 10 or 20 kPa) or super atmospheric oxygen concentration (40, 60 or 80 kPa) alone did not effectively prevent surface browning. However, dips of ascorbic acid (2%w/v), calcium lactate (1% w/v) and cysteine 0.5% w/v adjusted to pH 7.0 did significantly extend the shelf life of the pear slices.

Fresh fruits deteriorate as a result of respiration. In the absence of oxygen, anaerobic respiration occurs and generates off flavors, off-odors and metabolic tissue damage. This occurs when the partial pressure of oxygen drops to around 10 kPa (Lamikanra, 2002.). Gil et al. (1998) used low oxygen atmospheres below this limit with Fuji apples slices treated with ascorbic acid. The slices stored at 10 °C in 0.25 kPa of oxygen did not differ from the ones stored in air. The slices treated with 2% ascorbic acid and held in an atmosphere of 0 kPa oxygen at 10 °C did not show reduced visual quality or significant browning up to 15 days. The slices stored in 0 kPa of oxygen had higher reduced respiration and ethylene rates than the ones stored in air, but showed fermentative metabolites such as ethanol and acetaldehyde. However, these compounds still did not have a significant impact on off-flavor (Gil et al., 1998).

Carbon dioxide is the most important gas in MAP applications because of the inhibitory effect on microorganisms, and as temperature increases carbon dioxide protection against microorganisms decreases (Lamikanra, 2002). Poubol and Izumil (2005) used two cultivars of mango (Carabao and Nam Dokmai) to study the effect of high carbon dioxide atmospheres (3, 5 and 10%) at 5 and 13 °C. There were differences found by cultivar, freshly sliced Carabao cubes had lower respiration rate and total bacterial count, higher ascorbic acid content and firmness than Nam Dokmai. High carbon dioxide atmospheres retarded the development of water soaked appearance. Total bacterial count was lower in Carabao cubes during storage at both temperatures. At 10% carbon dioxide there were reduced bacterial counts for both cultivars but only for the ones stored at 13 °C. A 0% carbon dioxide atmosphere was recommended to reduce bacterial population when storage temperature abuse could occur.

Poubol and Izumi (2005) studied the quality of fresh cut mango cubes as affected by high oxygen controlled atmospheres. Atmospheres of 60 kPa of O₂ reduced the respiration of fresh cut Carabao mango cubes held at 5 °C or 13 °C. The high O₂ atmosphere of 60 kPa retarded the respiration rate slightly of Carabao mango cubes at 5 °C for 9 days and had no effects on the physiology and quality of Nam Dokmai mangos cubes at 5 °C for 4 days. At the higher temperature of 13 °C for 2 to 5 days, the high oxygen atmosphere caused an increase in respiration rate, browning, and growth of mesophilic aerobic bacteria on Carabao cubes and yeast grown on Nam Dokmai cubes. Because of these responses, application of high oxygen MAP was not recommended when mango cubes are stored at higher temperature than 5 °C. The reason might have been the higher pH of cubes stored in 60 kPa O₂ at 13 °C than at 5 °C or in air.

In a study by Bai et al. (2001) on cantaloupes, three different conditions were compared: 1. Fresh cut packages of cantaloupe were allowed to naturally form a modified atmosphere, 2. The internal atmosphere of the packages was flushed with a gas mixture of 4kPa of oxygen plus 10kPa Carbon dioxide and 3. The film was perforated with a needle to have ten 1.5 mm holes to maintain near atmospheric levels. Treatments 1 and 2 maintained the quality of melons for 9 days at 5 °C, treatment 2 maintained quality better than 1, as indicated by better color retention, reduced translucency, respiration rate and microbial population. Treatment 3 had a rapid decline as it showed tissue translucency and off-odor development.

Beaulieu et al. (2003) used passive MAP with fresh cut mangos of different ripeness (soft and firm) and different cultivars. Both Keitt and Palmer mango cubes stored in passive MAP clamshells at 4 °C had almost identical oxygen consumption rates that were independent of ripeness. After 4 days, packages had roughly 2.2-3.7 kPa O₂ and approached 0.1-0.4 kPa (anaerobic levels) by day 7. This system was inadequate to prevent anaerobic respiration after 7 days.

Another MAP method is called moderate vacuum packaging (MVP). The product is packed in a rigid airtight container under 40kPa of atmospheric pressure and kept at 4-7 °C. At the beginning, the gas composition is that of normal air (21% oxygen, 0.04% carbon dioxide and 78% nitrogen) but at a reduced partial gas pressure. The lower concentration of oxygen stabilizes the product quality by slowing down the metabolism of the produce and the growth of spoilage microorganisms (Alzamora, 2000).

Packaging films

There are different types of films that have shown benefit to fresh cut products. There are films with an adjustable “temperature switch” point, at which the film’s permeation changes rapidly. Landec Company uses long-chain fatty alcohol based polymeric chains that under predetermined temperature switch point the chains are in crystalline state, providing a gas barrier. But at the specified temperature, the side chains melt to a gas permeable amorphous state (Alzamora, 2000).

Other films that have been studied are made from two dissimilar layers or from two layers containing minute cuts and if the temperature rises or falls, the layers expand at different rates. Then, the film at the cut edge retracts and curls upwards to enlarged holes increasing the film permeability (Alzamora, 2000).

The oxygen transmission rates (OTR) of films are very important. However, films are required to meet other properties such as clarity, sealability, printability, stiffness and gas barrier. These combinations add to bring down the final OTR rates to 200-350 cc/m²/24 hr (IFPA, 2004).

Rapisarda et al. (2006) showed an example of how the permeability of films alone affects the shelf-life. They used fresh cut oranges packed with three different permeability films. Film A had permeability to oxygen of 35 cm³/m²/24 hour, film B had 56 cm³/m²/24 h and film C had 110 cm³/m²/24 h. The fresh cut oranges were stored at 4 °C for up to 15 days. All films did not show marked physicochemical changes on the fruit, but sensory data showed that fruit packaged with film C (the most permeable to oxygen) were the most appreciated because the film prevented the formation or removal of off flavors, yet they showed a higher reduction in the ascorbic acid content.

Fresh Hold is a concept in which a mineral, such as calcium carbonate, is diffused through a packaging film creating very small micro-pores throughout the structure. The pockets transfer oxygen and carbon dioxide through the film; this technology is owned by River ranch and is used in its fresh cut products (IFPA, 2004).

Other technologies that are considered to be used with fresh cut products are the use of antimicrobials incorporated into the packaging, two-way humidity control to continually adjust the internal package relative humidity, mold inhibitors incorporated in packages, carbon dioxide

release packages, and incorporation of desirable aromas in the packaging and time-temperature indicators to individual consumer packs (IFPA, 2004).

Wilson (2007) lists as active packaging for fruit and vegetables the following: packages with control of ethylene, packages with microbial control (sulfur and chlorine dioxide release), packages with active MAP, packages with humidity and condensation control and packages with odor control.

Edible Coatings

Edible coatings or enrobing fresh-cut product was investigated as a mechanism to apply a thin layer of protective material to the surface of the fruit (or vegetable) with the objective of replacing the natural protective tissue. Edible coating could help reduce respiration, retard water loss and water changes, improve texture and help retain volatile flavor compounds as well as reduce microbial growth. Edible coatings may be composed of polysaccharides, proteins, resins, waxes or oils (Lamikanra, 2002).

Lipid-based coatings are made from waxes and oils, such as paraffin wax or oil, beeswax, acetylated monoglycerides, stearic acid, lauric acid or sucrose fatty acids. These coatings are effective moisture barriers, whereas those containing resins (shellac, wood resin) are more permeable to water vapor. Some lipids and resins may cause anaerobic conditions at higher storage temperatures, whereas animal origin film can cause concerns by consumers such as vegetarians. Polysaccharides coatings such as cellulose, pectin, starch, alginates, chitosan, carrageenan and gums are good gas barriers and adhere well to cut surfaces. Their hydrophilic nature consequently leads to poor moisture barrier properties. Proteins such as casein, gelatin, soy, zein, and egg albumen are good film-formers and will adhere to hydrophilic surfaces, but in general, they do not resist water vapor diffusion (Baldwin et al., 1995).

Another important use of edible coatings in fresh cut products is to serve as carriers of ingredients that perform a specific function such as antimicrobial and fungicides agents. The edible coating should create a barrier that can retard the loss of desirable flavor volatiles and water vapor by restricting the exchange of carbon dioxide and oxygen (Baldwin et al., 1995).

Chitosan is capable of forming films or membranes. Zhao et al. (2004) used chitosan-based edible coatings with added calcium or Vitamin E on strawberries and raspberries stored either at 2 °C and 88% RH for 3 weeks or at -23 °C up to 6 months. The coatings significantly decreased decay and weight loss, and delayed the change in color, pH and titratable acidity, as well as reduced drip loss and better texture. The addition of calcium and vitamin E increased the content of these nutrients. Vitamin E content for one serving of strawberries increased from 4.6-6.6% (non-coated) to 22.7-39.9% Dietary reference intake (DRI) in coated fruit. Vitamin E content for raspberries increased to 78-102.3% of DRI whereas uncoated berries lost about 22 to 59% of their initial Vitamin E content. Coating treatment in strawberries increased calcium content to 6.8-7.3 % DRI and in raspberries 11% DRI.

Perez-Gago (2006) studied the effect of antioxidant type and content alone or in combination with edible coatings in fresh-cut apples. Edible composite coatings were prepared from whey protein concentrate (WPC) and beeswax (BW). Ascorbic acid (AA), cysteine (Cys), and 4-hexylresorcinol (4-hexyl) were incorporated in the formulations as antioxidants. Results showed that the incorporation of the antioxidant to the coating reduced browning compared to the use of the antioxidant alone. 4-hexylresorcinol was the least effective in reducing browning, even when incorporated into the WPC-based coating. Increasing AA and Cys content decreased browning of coated samples. The most effective treatments were WPC-BW-based coatings with 1% AA or 0.5% Cys. Coating application did not reduce weight loss in fresh-cut apples, probably due to the high relative humidity of the product. A sensory panel was able to discriminate between samples coated with WPC-Cys and samples dipped in Cys aqueous solution, but not between samples coated with WPC-AA and samples dipped in AA aqueous solution.

McHugh and Senesi (2000) used edible coatings and films made from apple puree combined with various concentrations of fatty acids, fatty alcohols, beeswax and vegetable oil to apply to apple pieces. Apple pieces were coated with the solutions or wrapped in the preformed films. Wraps were significantly more effective than coatings as results of changes in moisture content and color.

Lee (2002) used edible coatings of carrageenan or whey protein concentrate in fresh cut apple slices. The edible coatings were combined with antibrowning agents such as ascorbic acid, citric acid, oxalic acid and its combinations. Carrageenan coatings reduced the respiration of

apple slices by 10% and whey protein concentrate coatings by 20%. The best antibrowning treatment was ascorbic acid plus oxalic acid.

Olivas et al. (2003) used edible coatings composed of methylcellulose, stearic acid and additives (ascorbic acid, calcium chloride and sorbic acid) to preserve Anjou pear wedges stored at 4 °C and 78% RH during 12 days. The use of methylcellulose-stearic acid coatings prevented weight loss, whereas the methylcellulose alone showed poor water vapor barrier. The coatings did not have any effect on titratable acidity, soluble solids and microbial load and the use of additives retarded the appearance of browning.

Rojas-Grau et al. (2007) used edible coatings with essential oils such as lemongrass, oregano oil and vanillin incorporated in apple puree-alginate edible coatings on fresh-cut 'Fuji' apples. Coated apples were packed in air filled polypropylene trays and wrapped with polypropylene film during 21 days storage at 4 °C. A significant reduction in the rates of O₂ depletion and CO₂ production was observed in samples containing high concentrations of essential oils. Ethylene production in the coated apples remained below 50 µL/L⁻¹, whereas production of this gas increased continuously in uncoated apples and those coated without essential oils during storage. Apples coated with apple puree-alginate showed ethanol and acetaldehyde formation in the first week. Coatings with calcium chloride and N-acetylcysteine helped to maintain firmness and color, while lemongrass containing coatings induced severe texture softening. The most effective coating in terms of sensory quality after 2 weeks of storage was vanillin (0.3% w/w). All coatings significantly inhibited the growth of psychrotrophs aerobes, yeasts and molds. The antimicrobial effect of essential oils against *L. innocua* inoculated into apple pieces before coating and was tested. Lemongrass (1.0 and 1.5% w/w) and oregano oil containing coatings (0.5% w/w) exhibited the strongest antimicrobial activity against *L. innocua* (4 log reduction).

Albanese et al. (2007) used trehalose (alpha-linked disaccharide) as an edible coating on fresh cut "Annurca" apples slices stored at 6 °C. A trehalose 0.8% solution was compared with 1.0% sucrose and 0.1% sodium chloride. The results showed that trehalose reduced browning and reduced weight loss but loss of organic acids (ascorbic and malic acids) was observed.

Rojas-Grau et al. (2007) determined the effect of polysaccharides (alginate gellan-based) based edible coatings to keep the quality of fresh cut Fuji apples kept for 23 days at 4 °C. The edible coatings were effective up to 2 weeks of storage, but then there was ethanol and

acetaldehyde formation indicating fermentation. The edible coatings combined with the application of calcium chloride and N-acetylcysteine helped maintain firmness and color.

Vacuum Impregnation

Vacuum Impregnation is a technique used for enhancing the functionality of high porosity foods by filling the porous microstructure with desired solvents and solutes. The operation is done in two steps after the product is immersed in the tank with the liquid phase. The first step consists of the application of vacuum pressure and the second step of the restoration of atmospheric pressure in the tank (Alzamora et al., 2000).

Jeon and Zhao (2005) evaluated the antioxidant capacity of thirteen US Northwest honeys from different floral sources and their anti-browning effect on fresh-cut apples. Honey was applied to fresh-cut apples by simply immersing apple slices in 10% honey solution for 30 min and by vacuum impregnating (vacuum at 75 mmHg for 15 min followed with 30 min restoration at atmospheric pressure) in the same honey solution. The 10% diluted high-fructose corn syrup solution was used as a comparison. The surface color of the apple slices and physicochemical properties were monitored during 14 days of storage at 3 °C and 90% relative humidity. Vacuum impregnation with honey was more effective in controlling browning discoloration than that of simple immersion treatment. Honey in combination with vacuum impregnating operation may have a great potential for developing high quality fresh cut fruits.

Park et al. (2005) used vacuum impregnation for extending the shelf life and developing nutritionally fortified fresh-cut apples. The objective was to fortified fresh cut apples with vitamin E, calcium and zinc using a solution of 20% high fructose corn syrup (HFCS) or 1% of calcium caseinate. The treatment increased vitamin E more than 100 times, and calcium and zinc contents about 20 times compared with unfortified apples. Consumer sensory panel evaluated the apples in liking, color, texture and overall acceptance of HFCS-treated apples. Both HFCS and calcium caseinate were identified as good solutions for vacuum impregnation. There were changes in physicochemical properties of apples, but consumers did not identify these.

Osmotic Dehydration

Osmotic dehydration is considered a minimal processing treatment to preserve fresh-like characteristics of fruits or to treat products to be used as ingredients in others food products such as fruit ice cream and yogurts. Osmotic dehydration preserves attributes such as color, texture and aroma and reduces water activity (Moreno et al., 2004).

The small water activity reduction caused by osmotic dehydration allows incorporating additives such as preservatives and pH depressing agents (Pereira et al., 2004).

Pereira et al. (2004) treated guavas with osmotic dehydration and packed under passive modified atmosphere before storing at 5 °C. Guavas treated with osmotic dehydration were found to have significant changes in texture, but the color of the fresh fruit remained without change. There was a benefit on microbial conditions and color under modified atmosphere. All together, storage temperature, modified atmosphere packaging and osmotic dehydration maintained the quality of the guavas during 24 days.

Moreno et al. (2004) compared the effect of osmotic dehydration at atmospheric pressure and under vacuum impregnation using papaya and solutions of sucrose (55 and 65°Brix at 30 °C). Osmotic dehydration caused shape changes and size reduction of papaya cells; the vacuum impregnated samples showed some changes in the structure of cells, with largest firmness and overall texture improvement for the vacuum impregnated samples due to the greater thickness of the middle lamella.

Castello et al. (2005) used osmotic dehydration and vacuum impregnation in strawberry halves osmodehydrated using up to 30° Brix solutions to monitor the respiration rates. These treatments resulted in a decrease in oxygen consumption, but no notable changes in CO₂ generation. Ethanol and acetaldehyde were detected, showing that an anaerobic atmosphere was the dominant mode and not beneficial.

High Hydrostatic Pressure

This technology is the application of pressure uniformly throughout the product generated by heating of the pressure medium by direct or indirect compression. Microorganisms

and enzymes are affected by high hydrostatic pressure (HHP), whereas flavor and odor quality of certain food are retained. HHP is different from other non-thermal and thermal treatments in that the pressure generated can deform or noticeably modify fruit and vegetable integrity, especially when treating a porous product. This treatment affects texture and promotes other reactions such as enzymatic browning. Thus the application of this technology was used mostly on juices, jams, jellies, salsas, yogurt and dressings (Alzamora et al., 2000).

High pressure processing is called high hydrostatic pressure or ultra high pressure processing. HHP is used for liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa. Process temperature during pressure treatment can be specified from below 0 °C to above 100 °C, exposure times at pressure can range from a millisecond pulse to a treatment time of over 1200 s (FDA, 2000 (a)).

Aleman et al. (1999) compared the use of step-pulsed to static pressure treatments using inoculated fresh cut pineapples cubes with 10^4 - 10^5 CFU/g *Saccharomyces cerevisiae* packed in heat-sealed polyethylene pouches. Static treatments included 100 and 9000 s at 270 MPa and 9000 s at 340 MPa. Step-pulsed pressure treatments included 100, 300 and 600 s at 0-270 MPa using 0.5-s and 10-s pulses. All treatments were held at 4 °C for 60 days. Static treatment at 270 and 340 MPa for 9000 s resulted in <240 CFU/g yeasts and bacteria counts for up to 60 days. Step-pulsed pressure treatments for 100 s at 0-270 MPa using 0.5-s (200 pulses) and 10-s pulses (10 pulses) were more effective than a 100-s static 270-MPa treatment. This study confirmed the superiority of step-pulsed over static pressure treatments.

Lau et al. (2002) used high pressure (450 and 800Mpa) to treat apple slices in syrup (11% sucrose) with different antibrowning treatments. The antibrowning treatments were 0.5% citric acid, 0.5% copper (II) ascorbate or 90 ppm cysteine. The samples were stored at 4 °C for 10 weeks. The treatment with high pressure extended the shelf life of the apples to more than 10 weeks, while the control was less than 2 weeks. High pressure treatment without antibrowning turned the apples brown immediately after the treatment, but this effect was inhibited with the addition of ascorbate.

Radiation

Irradiation is defined as the emission and propagation of energy through space or medium. The electromagnetic radiation of interest in food preservation may be divided in microwaves, ultraviolet rays, X-rays and gamma rays. The most important radiations in food preservation are the electromagnetic radiations with wavelengths of 2000 Å or less (Jay, 2005).

The use of irradiation on fruit and vegetables was done with the objective of insect and disease disinfestations or delay of ripening and sprouting. Recently, radiation has been investigated for use in fresh cut products although the application of irradiation might cause undesirable biochemical changes, such as enzymatic browning (Lamiknara, 2002).

The response of fresh fruit respiration to irradiation depends on cultivar, maturity and irradiation dose levels. The effect of irradiation (0-11 kGy) on respiration and ethylene rates of apple slices was determined by Gurbuz et al. (2000) on different cultivars of apples and in a pre climacteric and post climacteric stage. The responses were variable depending on cultivar and physiological age. Respiration increased and ethylene production decreased in a dose dependent manner above 1.2 kGy. These higher rates were only at 4 hours post-irradiation and then reduced at 24 and 72 hours with even lower respiration rates than control (0 kGy), indicating that in the longer term the effect of irradiation may be minimal. At 2.4 kGy the effect on tissue physiology was minimal, but if used in combination with other preservation techniques the quality and safety of fresh cut apples was improved. Another limitation was that in some preliminary data doses above 0.4 kGy showed greater softening of apple slices.

Irradiation may be used in combination with other preservation methods to improve the quality or safety of fresh cut fruits. Xu et al. (2005) investigated the effect of low-dose ionizing radiation (gamma) and calcium ascorbate in fresh cut apple slices packed under modified atmosphere. The slices were treated with 7% calcium ascorbate and irradiated with 0.5 and 1.0 kGy followed by storage at 10 °C for up to 3 weeks. Calcium ascorbate reduced the loss of firmness and increased ascorbic acid retention. Calcium ascorbate increases ascorbic levels and the ascorbic acid may provide protection against the effects of irradiation but antioxidants may increase radiation resistance of microorganisms. However, calcium ascorbate did not influence the microflora population. Radiation at 1 kGy significantly reduced microflora population during the 3 weeks storage period considering that 10 °C storage temperature was

used to simulate a possible temperature. The combination of calcium ascorbate and irradiation enhanced microbial safety while maintaining quality of fresh-cut apples slices.

Xuetong et al. (2005) investigated the effects of calcium ascorbate and ionizing irradiation (gamma) on *Listeria monocytogenes* inoculated on Gala apple slices. As radiation dose increased, the population of *L. monocytogenes* decreased linearly during storage at 10 °C. The results showed that calcium ascorbate increased irradiation resistance of *L. monocytogenes* indicating that a higher dose is required to inactivate *L. monocytogenes* on apples treated with calcium ascorbate. To achieve a 5 log reduction of *L. monocytogenes* a dose of 1.6 kGy was required. The apple slices with this treatment were packaged in modified atmosphere and stored for 14 days at 4 °C. This treatment did not affect color, aroma, soluble solids, titratable acidity or pH of apple slices, but slightly reduced firmness. Calcium ascorbate reduced the loss of firmness resulting from the irradiation, but lowered the aroma intensity of apple slices regardless of irradiation. The use of 3.5% calcium ascorbate and irradiation preserved the quality and safety attributes of fresh cut apple slices.

Lamikanra et al. (2005) studied the effect of processing cantaloupe melon under ultraviolet light radiation before cutting and after cutting, followed by storage of the fresh cut product at 10 °C. Ultraviolet light treated melons before cutting increased peroxidase production relative to the post cut treated and untreated fruits as a defense mechanism. The treated melon before cutting showed reduced esterase activity, as well as loss of lipase activity and respiration rate. The results showed a benefit in treated fruit under Ultraviolet light-C light before cutting to improve sensory quality and shelf life as observed by lower microbial counts, improved firmness and reduced rancidity aroma.

Sterilizing doses of radiation are usually insufficient to destroy the enzymes in foods; therefore to be able to avoid undesirable post irradiation changes is necessary to destroy these enzymes with treatments such as blanching (Jay et al., 2005).

Xuetong et al. (2006) used a combination of hot water surface treatment of whole fruit and low dose gamma irradiation of fresh-cut cantaloupe cubes. The whole cantaloupes were washed with tap water at 20 °C or tap water at 76 °C for 3 minutes. The fresh cut cantaloupe cubes were prepared and packed in clamshell containers, exposing half of the samples to 0.5 kGy of gamma radiation. All samples were stored for 7 days at 4 °C. The results showed that the hot water surface treatment reduced the microflora by 3.3 Log on the surface of whole fruits. The

combination of surface hot water treatment and irradiation resulted in small further reductions (0.5 to 0.6) in the cut pieces. The differences were not significant in firmness among the treatments on any storage day. Overall, the firmness of melon cubes was not significantly affected by irradiation or hot water treatment because during the hot water treatment, only the surface and the tissues under the rind were heated and heat did not penetrate far enough into the flesh to affect the texture. The cubes prepared from melons treated with hot water (irradiated or not) tended to have lower ascorbic acid concentration (221 ug/ml) than did cubes prepared from melons treated with cold water (246.9 ug/ml).

Hajare et al. (2006) investigated the effect of radiation (2 kGy) on the nutritional and sensory quality of pineapple. Results showed that there was no difference on total vitamin C content after the treatment, but in both treatments (radiation and no radiation) there was a significant decrease with time during the 12 days of storage at 8-10 °C. Carotenoids were stable during the whole period for both treatments. Sensory evaluation indicated that there was not a significant difference detected in texture, color and flavor.

Sanitizers

Sanitizers are chemicals that may be used to reduce microorganisms from the surfaces of whole and cut produce since fresh produce can be a vehicle of viruses, parasites, spoilage bacteria, molds and yeast, as well as occasional pathogenic bacteria (Alzamora et al., 2000).

FDA (2001,a) in “Methods to reduce/eliminate pathogens from fresh and fresh cut produce” summarizes the uses of several sanitizers: chlorine (hypochlorite), chlorine dioxide and acidified sodium chlorite, bromine, iodine, quaternary ammonium compounds, acidic compounds with or without fatty acid surfactants, alkaline compounds (phosphates), peracetic acid alone or in combination with fatty acids and hydrogen peroxide. Some examples of the use of sanitizers for fresh cut fruits are discussed here.

Chlorine is an effective sanitizer for surfaces that may come in contact with fruits and vegetables during harvesting and handling, as well as processing equipment. Chlorine is commonly used at 200 ppm (free chlorine or concentration of hypochlorous acid) and at a pH below 8.0, with a contact time of 1-2 minutes. Temperature of chlorinated water should ideally be at least 10 °C higher than fruits or vegetables to achieve a positive temperature differential

and minimize uptake of wash water through tissues. Chlorinated water is widely used to sanitize whole fruits and vegetables and fresh cut produce (Alzamora et al., 2000). The use of chlorination as commonly used for fresh-cut salad sanitation, may not be desirable for all fresh cut fruits. Washing or dipping after cutting may cause negative consequences, such as the washing away of desirable flavor and increased water activity (Lamikanra, 2002).

Baldwin (2003) investigated the effect of a sanitizer, firming agent and reducing/antibrowning agent and film formers in fresh cut apple quality as a dipping solution. An aqueous solution with hypochlorite as a sanitizer; sodium erythorbate (isoascorbate), N-acetylcysteine and 4-hexylresorcinol as reducing and anti-browning agents; and Ca propionate as a firming agent was developed as post processing dip of fresh-cut Gala apple. The additional effect of edible coating materials to the aqueous solution of additives was investigated. The edible coating film-forming agents were soybean oil emulsion, chitosan and carboxymethyl cellulose (CMC), that were expected to form a protective layer on the cut surface of the apple wedges, decreasing water loss and other deteriorating factors due to cutting. Apple slices were dipped in aqueous solutions of sanitizer, with or without anti-browning and firming agents (additives), and with or without film-formers. Treated slices were packed in perforated polyethylene bags and stored at 5.5 °C for up to 14 days. Slices dipped in water (containing hypochlorite only) lost marketable quality within a day, because of severe browning. Slices dipped in the aqueous solution plus additives maintained cut surface color, inhibited ethylene production, maintained firmness, and maintained the major aroma of apple. Addition of soybean oil emulsion reduced water loss, whereas chitosan and CMC did not, although water loss was not a problem for polyethylene-packaged products. These results suggest that a dip with a sanitizer, firming agent, and reducing/anti-browning agents is beneficial of fresh-cut apple quality. Addition of film-formers did not reduce decay as has been reported for whole fruits.

Chlorine dioxide is used as a sanitizer and is less affected by pH and organic matter, and less corrosive. Chlorine can be used at a maximum of 200 ppm and can be used for washing whole fresh fruits and vegetables at a concentration of 3 ppm or less. FDA does not permit the use of ClO₂ to sanitize other freshly cut fruit and vegetables (Alzamora et al., 2000). Plotto et al. (2004) investigated use of chlorine dioxide for fresh cut mangoes with other treatments of polysaccharides coatings and treated pieces maintained quality better than controls.

Organic acids are used as antimicrobial acidulants to preserve foods. The antimicrobial action is due to pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH. Fruits contain naturally significant concentrations of organic acids such as acetic, benzoic, citric, malic, sorbic, succinic and tartaric acids, but other such as melons and papayas contain lower concentrations. Treatment with citric acid in the form of lemon juice has been shown to reduce population of *Salmonella Typhii* inoculated on cubes of papaya (FDA, 2001 (b)).

Wan et al (2006) investigated the inactivation of *E.coli* 0157:h7 with peroxyacetic acid, acidified electrolyzed water and chlorine on cantaloupes and fresh cut apples. Electrolyzed water is generated from the reaction of Cl_2 and water in an anode site when NaCl solution (<10%) is electrolyzed through a septum; this results in acid water (Izumi, 1999). They concluded that all sanitizers showed a significantly higher inactivation than the controls. But the peroxyacetic acid treatment was more effective than the other sanitizers.

Hydrogen peroxide has bactericidal activity because of the capacity to generate hydroxyl radicals. Ukubu et al. (2005) used hydrogen peroxide in combination with nisin, sodium lactate and citric acid to reduce the bacterial transfer of pathogens from whole melons surfaces to fresh-cut pieces. They inoculated whole cantaloupe and honeydew melons with *E. coli* 0157:H7 and *listeria monocytogenes* and concluded that treatments reduced transfer of spoilage bacteria and pathogens from melon rind to the flesh.

Sapers et al (2001) evaluated the efficacy of antimicrobial treatments with sodium hypochlorite, H_2O_2 , commercial detergent formulations and trisodium phosphate. Cantaloupe samples were held at 4 °C. Washing with 5% H_2O_2 or with a commercial detergent formulation followed by 5% H_2O_2 at 50 °C was more effective than washing with water, surfactant solutions, 1000 ppm Cl_2 , trisodium phosphate or commercial detergents in reducing the microbial load on cantaloupe rind.

Ozone has been used for the treatment of water due to its strong oxidizing power and has been investigated to decontaminate various types of foods. Since ozone is a very strong oxidizer, in the case of bananas, it can result in physiologic injury at concentrations of 1.5ppm, showing black spots on the skin; although on oranges, strawberries, raspberries, grapes, apples and pears ozonated water can extend the shelf life. Kim. et al. (2006) used cold ozonated water for washing fresh cut lettuce inoculated with *Escherichia coli*, *Salmonella enterica*, *Listeria*

monocytogenes and *Staphylococcus aureus*. All pathogenic bacteria decreased by 99% within 1 minute of treatment with 5mg/L ozonated water and for total cell counts the reduction was 3-4 log similar to the results of 100ml/L chlorinated water.

Other Antimicrobials

Calcium propionate is a widely used food antimicrobial. Saftner et al. (2003) used solutions of hypochlorous acid with or without calcium propionate, calcium chelate or CaCl₂ on fresh cut honeydew chunks stored at 10 °C. Microbial development was higher on non treated melon samples than on hypochlorous plus calcium propionate treated samples. Sensory preference was not detected among hypochlorous, hypochlorous plus calcium propionate, or hypochlorous plus chelates treated samples and calcium salt and chelate inhibited changes in melon firmness.

Plotto et al. (2006) studied the effect of ethanol vapor prior to processing to extend the fresh cut mango storage with the objective of inhibiting ripening; finding that ethanol vapor applied for 20 hours to whole mangoes prior to processing for fresh cut is not a practical approach to delay ripening but at lower doses (10 hours) could be used as a safe microbial control for fresh cut products.

Carvacrol is a major component of the essential oil of oregano and thyme, and cinnamic acid occurs in cinnamon, cloves, black pepper, coriander and turmeric. Roller and Seedhar (2002) used these two compounds to inhibit microbial growth in fresh cut melon and kiwifruit stored in sealed jars at 4 and 8 °C. The treatment of fresh cut kiwifruit in carvacrol solutions of 5-15 nM reduced total viable counts from 6 to <2 log CFU/g for 21 days at 4 °C, but there was presence of undesirable color and odors. Treatments with 1nM of carvacrol or cinnamic acid delayed spoilage of fresh cut kiwifruit and honeydew melon without the adverse sensory consequences observed at higher concentrations.

Wang and Buta (2003) used methyl jasmonate and other volatiles to study the effect on kiwifruit fresh cut slices placed in polystyrene trays kept at 10 °C. The volatile compounds were introduced inside the trays before covering with lids. The use of methyl jasmonate (2.24, 11.2 or 22.4 ul/L) was effective in keeping the quality for 3 weeks. Similar results were obtained with

absolute ethyl alcohol (300 ul/L) or isopropyl alcohol (300 ul/L). 1-Propanol was less effective and methyl alcohol was not effective.

Andres et al. (2004) used fresh orange juice with citric and ascorbic acids and potassium sorbate to pack red delicious apple cubes with plastic films of different gas permeabilities. They studied the growth of yeast and molds at storage temperatures of 4, 10 and 20 °C to determine the time required to reach microbial counts of 10⁶ CFU/g. At 4 °C, the stability of the fruit packed in natural juice only and with preservatives was more than 25 days. The use of a low gas permeability film and of potassium sorbate (0.125g/kg with citric and ascorbic acids) extended shelf life at higher temperatures.

Vasantha et al. (2006) studied vanillin, which is hydroxy-3-methoxybenzaldehyd. The functional groups of vanillin acting as antimicrobials are aldehyde, ether, and phenol. The inhibitory effect was tested against four pathogenic organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Salmonella enterica* and four spoilage organisms: *Candida albicans*, *Lactobacillus casei*, *Penicillium expansum* and *Saccharomyces cerevisia*. They used fresh cut apple stored at 4 °C for 19 days. The concentrations of vanillin to inhibit the microorganisms varied between 6 and 18 mM depending on the microorganims. 12 mM vanillin inhibited the total aerobic microbial growth by 37% in fresh cut Empire apples and 66% in Crispin apples. Vanillin did not affect the control of enzymatic browning and softening by Nature Seal (calcium ascorbate).

Biopreservation

Biopreservation has been used to gain increased control of the growth of spoilage and pathogenic bacteria, especially toward the end of the shelf life of fresh cut with or without modified-atmosphere packaging. Biopreservation uses mainly lactic acid bacteria (LAB). LAB can inhibit or eliminate the growth of many different microorganisms, including bacteria, yeast, and fungi, through the production of organic acids, diacetyl, hydrogen peroxide, enzymes, defective phages, lytic agents and antimicrobial peptides or bacteriocins (Alzamora et al 2002).

Vescovo et al. (1995) studied the addition of selected lactic acid bacteria strains and their inhibitory effect on the growth microflora associated with ready-to-use vegetables during

refrigerated storage. In particular, *coliforms* and *enterococci* were strongly reduced or eliminated from the products from the third day of storage. *Lactobacillus casei* strains proved more effective than *pediococci*. The use of lactic cultures able to produce bacteriocins and to grow at low temperatures could be a useful tool to preserve fresh vegetables and to ensure their microbiological safety

Leverentz et al. (2003) found that inoculated *Listeria monocytogenes* populations survived and increased only slightly on fresh-cut Red Delicious apples stored at 10 °C but increased significantly on fresh-cut honeydew melons stored at 10 °C during 7 days. They studied the effect of lytic *L. monocytogenes*-specific phages via two phage application methods, spraying and pipetting, on *L. monocytogenes* populations in artificially contaminated fresh-cut melons and apples. The phage mixture reduced *L. monocytogenes* populations by 2.0 to 4.6 log units over the control on honeydew melons. On apples, the reduction was below 0.4 log units. Nisin is a bacteriocin produced by lactic acid bacteria approved as a preservative in fifty countries and the only purified bacteriocin that is commercially available. The activity of nisin increases at pH below 5 and this make nisin suitable for use on fruit. The treatments of the phage mixture with nisin reduced *L. monocytogenes* populations by up to 5.7 log units on honeydew melon slices and by up to 2.3 log units on apple slices compared to the control. Nisin alone reduced *L. monocytogenes* populations by up to 3.2 log units on honeydew melon slices and by up to 2.0 log units on apple slices compared to the control. The phage titer was stable on melon slices, but declined rapidly on apple slices. In conclusion, the spray application of the phage and phage plus nisin reduced the bacterial numbers at least as much as the pipette application. The effectiveness of the phage treatment also depended on the initial concentration of *L. monocytogenes* and both; phage and nisin applications reduced pathogenic bacterial contamination and growth on produce.

Laverentz et al. (2006) studied the of control *Listeria monocytogenes* on honey dew melon pieces using a treatment consisting of either *Gluconobacter asaii*, a bacterial antagonist naturally occurring on the pome fruit, or a bacteriophage mix or a combination of the two. The bacteriophage mix and *G. asaii* were effective antagonists of *L. monocytogenes* alone, but the combination was even more effective. *G. asaii* alone reduced populations approximately 3-4 logs and phage alone reduced populations by one log compared to the *L. monocytogenes* control over 7 days. The combination reduced *L. monocytogenes* populations by one log by day 2 and up to 6

logs by day 7. The results showed that bacteriophages had an immediate inhibitory effect, while *G. asaii* offered longer term control. *G. asaii* was effective because it competes for space and nutrients on the fruit surfaces where the bacteria would otherwise exist. The bacteriophages invade bacteria and damage walls, which allows more to invade and cause further damage.

Martin-Diana et al. (2006) studied the use of whey permeate, a by-product of cheese processing, as a natural bio sanitizer. The concentrations used were 0.5, 1.5 and 3% as a washing treatment of fresh cut carrots and lettuce. Whey permeate at 3% resulted in lower or equivalent microbial load than treatment with chlorine (120 ppm). Although a sensory panel evaluated the lettuce as acceptable, it was not the same for the carrots.

Other Technologies

These technologies are not currently used but could possibly have some potential for use in fresh cut products in the future.

High Intensity Pulsed Electric Fields (PEFs)

High intensity pulsed electric fields consist in the application of short pulses of high voltage into food materials. This technology is better suited for liquid foods where food can be effectively cooled as continuously flows between treatment electrodes (Hoover, 1997).

Microbial inactivation by this technology has been proven to be a function of the particular electric field and number of pulses applied but also other factors as pulse wave shape, frequency, product composition, product physical and chemical characteristics, processing temperature and type of target microorganisms (Alzamora et al., 2000).

Toepfl et al. (2006) reviewed the use of high intensity pulsed electric fields for preservation of foods as an interesting alternative to traditional techniques like thermal pasteurization. Inactivation studies with three bacteria (*E. coli*, *Bacillus megaterium*, *Listeria innocua*) and the yeast *Saccharomyces cerevisiae* were performed using parameters such as field strength, total pulse energy input and treatment temperature. This study found that temperatures

higher than 40 °C can strongly increase the lethality of the PEF process and that small cells like *Listeria* are easily affected by pulsed fields even at a field strength as low as 16 kV cm⁻¹.

In relation to the possible application to fresh cut fruit, Angersbach et al. (2000) studied the effect of direct current fields on the cells of potato, apple, fish tissues and plant suspension cultures. A slight membrane breakdown phenomenon occurred in the first few microseconds after the start of the pulse at a critical electric field strength of 150-200V/cm and significant membrane breakdown was observed when the field strength of the electric pulses applied directly on the cell systems was in the range of 400–800 V/cm.

Pulsed electric field is one of the more promising non thermal processing method inducing membrane permeabilization within a very short time (µs to ms range) leaving the product matrix without changes while positively affecting mass transfer in subsequent processing of foods. The state of cell membrane systems should be determined to minimize cell damage in minimal processes, monitoring disruption for mass transfer purposes and inducing biosynthetic stress/wound reactions and responses (Ade-Omowaye et al., 2001).

Oscillating magnetic Fields

Magnetic fields affect the growth of microorganisms and under proper circumstances have the potential to pasteurize foods but their application has been limited by variable results and product thickness limitations (Hoover, 1997).

Static (SMF) and oscillating (OMF) magnetic fields have been studied for their potential as microbial inactivation methods. For SMF, the magnetic field intensity is constant with time, whereas an OMF is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. The magnetic field can be homogeneous or heterogeneous. Homogeneous magnetic fields have uniform field intensity in the area enclosed by the magnetic field coil. Heterogeneous fields have non-uniform field intensity, with the intensities decreasing as distances from the center of the coil increases. Oscillating magnetic field applies pulses and reverses the charge for each pulse, and the intensity of each pulse decreases with time to about 10% of the initial intensity (FDA, 2000 (a)).

Preservation of foods with OMF is done by sealing food in a plastic bag and applying a treatment of 100 pulses in an OMF with a frequency between 5 to 500 kHz at temperatures in the

range of 0 to 50 °C for a total exposure time ranging from 25 to 100 ms. Frequencies higher than 500 kHz are less effective for microbial inactivation and tend to heat the food material (FDA, 2000 (a)).

Food products under a magnetic coil and subjected to one or more pulses of an oscillating magnetic field having an intensity of between about 2 and about 100 Tesla and a frequency of between about 5 and about 500 kHz and a single pulse of the magnetic field generally decreases the microorganism population by at least about two orders of magnitude (Hofmann, 1985).

High Intensity Pulsed Light

This technology depends on a rapid, intense, magnified flash of light or electrical energy from a capacitor. The antimicrobial effects are greater than those of non-pulse or continuous wave conventional UV irradiation (Hoover, 1997).

The use of a pulsed light source in the UV range was evaluated (Lagunas-Solar et al., 2006) as a surface disinfection on fresh whole fruits (apple, kiwi, lemon, nectarines, oranges, peaches, pears, raspberries and grapes); the fruits were inoculated with fungal pathogens, the samples inoculated only showed a partial disinfection because of surface irregularities. For maximum disinfection was necessary to combine the source of light with dispersing reflectors and that the fruits must be handled in a way that ensures exposure to multidirectional incident beams.

Marquenie et al. (2003) evaluated the combination of pulsed white light, heat treatment and UV light as a treatment for surface decontamination for strawberries that were inoculated with conidia of *Botrytis cinerea*. For a period of 10 days, fungal development and structural damage were evaluated. Pulsed light treatments consisted of 30 μ s pulses at a frequency of 15 Hz for a total duration of 40 to 250 seconds. For the combination experiments, the UV doses were 0.5 or 1.0 kJ/m and the heat treatments were at 40 and 45 °C for 3 or 15 minutes. Pulsed light treatments alone had no positive or negative effect. The combination of a thermal and an UV treatment permitted a decrease of the thermal treatment to 40 °C for the same level of fungal inactivation. No external fruit damage was reported at this temperature. A combination of a thermal treatment with light pulses did not result in a significant difference in fungal growth.

Combining two illumination treatments did not cause a significant decrease in fungal development.

Ultrasound

Power ultrasound is used at frequencies in the range 20-100 kHz and requires the presence of a liquid medium for power transmission. The bactericidal effect of ultrasound is generally attributed to intracellular cavitation. The micro-mechanical shocks are created by making and breaking microscopic bubbles that are induced by fluctuating pressures under the ultrasonication process. These shocks disrupt cellular structural and functional components up to the point of cell lysis (FDA, 2000 (a)).

Seymour et al. (2002) investigated the effectiveness of power ultrasound (25 to 70 kHz) for the microbial decontamination of minimally processed fruit and vegetables and fruits (iceberg lettuce, whole cucumber, cut baton carrot, capicum pepper, white cabbage, spring onion, strawberry, curly leaf parsley, mint and other herbs). Each item was cut or uncut and inoculated with *Salmonella Typhimurium*, *Listeria monocytogenes* and *E. Coli* and washed for 10 minutes with water only, with chlorine solution (25ppm), ultrasound treatment or ultrasound treatment combined with chlorine solution (25ppm). In general, if the surfaces were cut the log reductions achieved were lower than for the uncut surfaces; suggesting that bacterial attachment is greater for the cut products compared with the corresponding uncut products for *Salmonella* but not for *E coli* and *Listeria monocytogenes*. The treatments and the food items were statistically significant from each other. The chlorine wash was significantly better than the tap water only treatment but there were no other significant differences between treatments. The cabbage was the most easily washed (2.8 Log reduction) and parsley was the least easily washed (0.8 log reduction). The frequency of ultrasound treatment had no significant effect on decontamination efficiency because the average reductions ranged from 1.3 to 1.4 at the different frequencies and the no ultrasound control had a reduction of 1.0 log. Although there was one additional log reduction by ultrasound plus a chlorinated water wash, this does not completely eliminate the risk of pathogens and with the potentially high capital expenditure for equipment and water treatment the authors concluded that it is unlikely that the fresh produce industry will be willing to adopt the ultrasound technology.

Conclusions

Fresh cut processing damages the fruit tissue inducing many physiological disorders that cause texture loss, browning and microbial load increase; this makes the application of one or combination of treatments necessary to minimize the changes.

The application of the different technologies to fresh cut fruit processing will not be successful if the preharvest and harvest factors are not considered equally important factors as postharvest treatment.

There is a great correlation between preharvest with postharvest physiology and response of fruit to fresh cut processing, which presents an area for further research and study.

This review summarizes the main technologies for processing fresh cut fruit to minimize the consequences by wounding, although there is still a need for more research in the areas of new non thermal technologies that could have potencial for application in fresh cut processing.

The trend towards the use of natural compounds to treat fresh cut products might present an area for further research since the consumer would prefer these compounds over other compounds.

Treatments to increase the nutritional quality of the fresh cut fruits presents an area of opportunity for fresh cut processors to deliver an advantage over fresh fruit; trend that is supported by the consumer's demand to have richer or equal products to fresh fruits.

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